Virologisches Institut
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. sc. nat. ETH Cornel Fraefel

Arbeit unter wissenschaftlicher Betreuung von
PD Dr. med. vet. Claudia Bachofen

The diversity and dynamics of the bovine microbiome in Swiss dairy cows

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Karin Meier

Tierärztin
von Uster, ZH

genehmigt auf Antrag von

PD Dr. med. vet. Claudia Bachofen, Referentin
Prof. Dr. med. vet. Michael Hässig, Korreferent

2020

Content

Zusammenfassung

Zunehmende Herdengrössen und ein Anstieg im Tierverkehr stellen hohe Anforderungen an Kontrolle und Management der Tiergesundheit. Prävention und Behandlung von Krankheiten bedingen grosses Wissen um die Zirkulation potenzieller Pathogene.

Das Ziel der Studie war es darum, die Veränderungen im Bakteriom und Virom von Milchkühen nach einer Herdenzusammenführung zu untersuchen. Zu diesem Zweck wurden Nasentupfer und Kotproben von drei Herden, die zu einer 120-köpfigen Herde zusammengeführt wurden, vor und 6 Monate nach dem Einzug in einen neu erbauten Stall gesammelt und mittels Next Generation Sequencing untersucht. Dabei zeigte sich, dass die Zusammensetzung und Diversität des fäkalen Bakterioms zwischen verschiedenen Proben relativ ähnlich war, während das nasale Bakteriom grössere individuelle Unterschiede aufwies. Sowohl im Kot wie in den Nasentupfern war der Unterschied zwischen verschiedenen Proben grösser als zwischen denselben Proben vor und nach der Herdenzusammenführung. Es wurden deutlich weniger virale als bakterielle Sequenzen gefunden und auch der Unterschied zwischen vor und nach Herdenzusammenführung war kleiner.

Zusammenfassend kann gesagt werden, dass in gesunden, adulten Milchkühen nur wenig virale Sequenzen nachweisbar sind und dass das Virom relativ stabil zu sein scheint. Im Gegensatz dazu war das Bakteriom diverser und reagierte, v.a. im Kot, stärker auf die Herdenzusammenführung, wobei die Art der Veränderung durch nicht-identifizierte individuelle Faktoren bedingt war.

Schlüsselwörter: Next Generation Sequencing, Milchkuh, Mikrobiom, Bakteriom, Virom

Abstract

Increasing herd sizes and animal movements intensify the exchange of microbiota in livestock animals. Awareness of potential pathogens circulating within a herd is essential for prevention and treatment.

The aim of this study was to compare the bacteriome and virome of dairy cows before and after herds are intermingled. This knowledge can provide novel information on how bacterial and viral communities change if herds are mixed. Three independent dairy herds were united to one herd of around 120 cows in a new stable. Of each cow a nasal swab and a faecal sample were taken before and 6 months after the herds were brought together and analysed by Next Generation Sequencing. The faecal bacteriome was similar between samples, whereas the established nasal bacteriome was more sample specific. In both sample types the differences in bacteriome composition between different samples was larger than between the same samples upon consolidation of the herds. Compared to the bacteriome, relatively few viral reads were detected and less changes observed upon consolidation of the herds.

In conclusion, we found that there were relatively low numbers of viral reads present in the nasal cavity and the gut of healthy adult dairy cows and that the virome, once established, was quite robust. In contrast, the bovine bacteriome was more diverse and, particularly in the gut, also more susceptible to environmental changes. The individual factor(s) driving the changes could not be identified.

Keywords: Next Generation Sequencing, dairy cow, microbiome, bacteriome, virome

1 Introduction

Infectious diseases account for huge economic losses in cattle industry. E.g. in 2011, 96.9% of the feedlots in the United States were affected by respiratory diseases and 70.6% by digestive problems (USDA–APHIS–VS, 2013). Therefore, losses only for treating sick animals are estimated to be USD $54.12 million a year in the United States (Johnson and Pendell, 2017). This shows the importance of awareness of infections pathogens, as they can have a grave influence on animal health and economics.

To put the numbers above in perspective the livestock population needs to be considered. The United States counted 94.4 million head cattle in the beginning of 2018 (USDA, 2018), which is 0.29 animals per capita (https://www.census.gov/popclock/, March 30, 2020). In comparison, the cattle population in Switzerland consists of 1,543,345 animals (https://www.bfs.admin.ch/bfs/en/home/statistics/agriculture-forestry/farming.html, November 19, 2019), which is 0.18 animals per capita (Bundesamt für Statistik BFS, 2019). Therefore, compared to the US, economic loss due to infectious diseases in Switzerland is most likely less extensive but overall animal health is still of great interest. In the year 2018, Switzerland was free of major cattle diseases, like bluetongue disease, bovine spongiform encephalopathy, brucellosis, enzootic bovine leucosis, infectious bovine rhinotracheitis, contagious bovine pleuropneumonia, foot and mouth disease, rinderpest, rabies, tuberculosis and vesicular stomatitis. The majority, more precisely 99%, of the cattle farms were free of bovine viral diarrhoea (Federal Food Safety and Veterinary Office, 2018). This attests a very good overall health to the Swiss bovine population. However, the most common disease in beef cattle are respiratory problems (Kälbergesundheitsdienst, 2018), while in dairy farming reproductive disorders, hoof diseases and mastitis are the major diseases affecting the animals (Federal Food Safety and Veterinary Office, 2015). The actual causative agent(s) remain often undiagnosed, particularly in case of the respiratory problems. This shows the need for research to identify the responsible pathogens.

Since the discovery of the DNA structure in 1953 (Watson and Crick, 1953) many attempts were made to determine the exact nucleotide sequence of DNA (Heather and Chain, 2016). A breakthrough was made in 1977, when the first full-length DNA genome, the one of the E. coli bacteriophage ϕX174 (PhiX), was successfully sequenced (Sanger et al., 1977). The method developed by Sanger as well as the method developed by Maxam and Gilbert are referred to as First Generation Sequencing (Heather and Chain, 2016). In the following years the method was constantly developed in order to allow more sequencing throughput, greater consistency and

reduce costs. To achieve this, an increasing amount of steps was performed by automated and robotic systems (Hunkapiller et al., 1991). Nevertheless, Sanger sequencing still has several disadvantages. For sequencing specially labelled nucleotides are used and for visualization electrophoresis is needed (Heather and Chain, 2016). It is also quite time consuming as there is only one sequence obtained per reaction (Kumar et al., 2019). The maximal possible sequence length is a few1000 base pairs (Jia et al., 2014). In order to overcome the limitations of Sanger sequencing, new methods were developed. These sequencing techniques are commonly related to as Next Generation Sequencing (NGS). They share the characteristics that the sequencing process is much faster than Sanger sequencing, and, thanks to the possibility of massive parallel sequencing, the data output is tremendously higher (Kumar et al., 2019).

The development of the pyrosequencing method (Hyman, 1988; Nyrén, 1987; Nyrén and Lundin, 1985) allowed the offset of a new sequencing era. This new approach allows real-time DNA sequencing with natural nucleotides. Thus, it eliminates the need for modified nucleotides and time-consuming electrophoresis for visualization (Ronaghi et al., 1996; Ronaghi et al., 1998). This pyrosequencing technique was implemented in high-throughput mass parallel sequencing machines, which produced an approximately 100x higher output than Sanger sequencing at that time (Margulies et al., 2005). This started off the age of Second Generation Sequencing with more parallel sequencing techniques being developed in the following years (Heather and Chain, 2016). A crucial step of library preparation of Second Generation Sequencing methods is DNA fragmentation and clonal amplification, which is a well-known source of errors and biases (Harris et al., 2008). These methods are also referred to as short-read sequencing, as they only achieve a read length of a few hundred base pairs (Kumar et al., 2019).

Sequencing methods, which overcome these disadvantages by being able to sequence single molecules and long-read sequencing, can be related to as Third Generation Sequencing (Heather and Chain, 2016; Kumar et al., 2019). From 2003 on, first Third Generation Sequencing methods for obtaining sequence information from single molecules were demonstrated (Braslavsky et al., 2003; Greenleaf and Block, 2006; Harris et al., 2008). Current state of the art Third Generation Sequencing machines can produce read length of over thousand base pairs (Kumar et al., 2019).

The Human Genome Project conducted with First Generation Sequencing methods took 10 years, international collaboration and cost 2.7 billion US dollars. With NGS this can be accomplished in three days in one laboratory for 1000 US dollars (Kumar et al., 2019). The

development of NGS techniques changed prizes per sequenced base dramatically. Before the time of NGS, the number of bases sequenced per US dollar doubled every 19 months. From 2004 on, with the development of NGS techniques, the doubling time was reduced to 5 months. In comparison, the doubling time of the amount of megabytes per US dollar is doubling constantly every 14 months since 1990 (Stein, 2010).

Different NGS platforms vary in their read size, number of sequenced reads and amount of data generated in a run. Disregarding the platform, there are certain common preparation steps. The basic three steps are sample preparation, nucleic acid sequencing and data analysis. Sample preparation includes DNA fragmentation, adapter ligation and DNA amplification in case of short-read sequencing. In the case of long-read sequencing the last step is omitted. Following sequencing, the data are analysed either by aligning the reads to one or many known sequence(s) (reference based assembly) or by aligning them to each other (*de novo* assembly) (Kumar et al., 2019).

With the revolution of NGS the field of genomic research greatly expanded. The continuous development and improvement of NGS techniques allows even relatively small labs to have access to a DNA and RNA sequencing facility. NGS led to a revolution in many research sectors. It is widely used in basic science, clinical and forensic research, disease diagnostics and metagenomics. Metagenomics is the study of genetic material and therefore the microbiome in a given environmental sample (van Dijk et al., 2014). Metagenomic studies repeatedly helped to identify emerging diseases where the causative pathogen was unknown. For example, in 2007, a study identified the Israeli acute paralysis virus of bees as the possible pathogen resulting in colony collapse disorder, which is characterized as a loss of workers in a bee colony (Cox-Foster et al., 2007). Another example is the identification of Schmallenberg virus. In 2011, an unknown disease caused fever, decreased milk production and diarrhoea in dairy cattle in Germany and the Netherlands. With a metagenomic approach a yet unknown orthobunyavirus was identified in blood samples of infected animals. The study could further prove that this newly identified virus, called Schmallenberg virus, is the causative agent of this new disease (Hoffmann et al., 2012).

In the current study the microbiome is defined as the microbial community found in an organ system. This includes the bacteriome, the total of bacterial genomes, and the virome, the entirety of viral nucleic acids, present in an organ system, in an individual or in any specified environment. Other microbial organisms such as fungi or parasites are also part of the microbiome but are not focused on in this study.

Data on the bacteriome of cattle are available for different organ systems. In the gastrointestinal tract the ruminal and gut flora is of interest due to its important role in bovine metabolism and development of disease (Gomez et al., 2017; Gruninger et al., 2019; Hagey et al., 2019; Henderson et al., 2015; Hu et al., 2019; Kim et al., 2014; Kim et al., 2017; Mao et al., 2015; Mao et al., 2012; Myer et al., 2015; Stewart et al., 2018; Sun et al., 2019; Thomas et al., 2017). In the reproductive system the bacteriome of the uterus and vagina (Ault et al., 2019a, b; Bicalho et al., 2017; Clemmons et al., 2017; Galvao et al., 2019; Machado et al., 2012; Miranda-CasoLuengo et al., 2019; Moore et al., 2017; Wang et al., 2018b) has been studied as well as the bacterial diversity of the mammary gland and milk (Addis et al., 2016; Andrews et al., 2019; Bhatt et al., 2012; Bonsaglia et al., 2017; Cremonesi et al., 2018; Derakhshani et al., 2018a; Derakhshani et al., 2018b; Doyle et al., 2017; Falentin et al., 2016; Ganda et al., 2016; Ganda et al., 2017; Hoque et al., 2019; Kuehn et al., 2013; Metzger et al., 2018; Tong et al., 2019). Especially in beef farming, the respiratory bacteriome is very much in focus due to the frequent respiratory infections (Gaeta et al., 2017; Holman et al., 2015a; Holman et al., 2015b; Holman et al., 2017; Johnston et al., 2017; Klima et al., 2019; Lima et al., 2016; McDaneld et al., 2018; McMullen et al., 2018, 2019; Stroebel et al., 2018; Timsit et al., 2016; Timsit et al., 2018; Zeineldin et al., 2019; Zeineldin et al., 2017a; Zeineldin et al., 2017b). Metagenomic studies have shown a distinctive transformation of the nasopharyngeal bacteriome of beef cattle upon transportation to a feedlot (Holman et al., 2017; Timsit et al., 2016). So far, the evolution of the nasal microbiome seems herd specific, as no common evolutionary pattern could be observed (McMullen et al., 2018).

There is considerably less data available on the virome of dairy cows (Kaszab et al., 2020; Kwok et al., 2020). Studies are published on the respiratory tract (Blomström et al., 2017; Hause et al., 2015; Mitra et al., 2016; Ng et al., 2015a; Tokarz et al., 2015; Zhang et al., 2019a; Zhang et al., 2019b) , on blood (Baechlein et al., 2015; Cibulski et al., 2016; de Souza et al., 2018; Hoffmann et al., 2012; Sadeghi et al., 2017; Wang et al., 2018a), on the central nervous system (Bouzalas et al., 2014; Li et al., 2013; Moreira et al., 2017; Schlottau et al., 2016; Wüthrich et al., 2016), on the gastrointestinal tract (Guo et al., 2018; Masuda et al., 2014; Nagai et al., 2015a; Nagai et al., 2015b; Otomaru et al., 2016) including the rumen (Anderson et al., 2017; Berg Miller et al., 2012; Ross et al., 2013), on the genital tract (Bauermann et al., 2017; Ling et al., 2019) and on the virome of milk (Hoque et al., 2019). For example, it has been shown that the bovine rumen microbiome has a core viral population which is relatively stable and another

part of the virome which is influenced by feed and therefore changes upon change of diet (Anderson et al., 2017).

To date there are no studies published regarding the bacteriome or the virome of healthy Swiss dairy cows.
In 2018, 564,190 dairy cows lived in Switzerland on 25,800 farms (https://www.bfs.admin.ch/bfs/de/home/aktuell/neue-veroeffentlichungen.assetdetail.8467547.html, November 25, 2019) and made up for 36.6% of the cattle population. The same year, the gross value of milk production in Switzerland was 2.2 billion Swiss francs, which highlights the importance of dairy production for the Swiss economy (https://www.bfs.admin.ch/bfs/de/home/aktuell/neue-veroeffentlichungen.assetdetail.9927607.html, November 25, 2019). Therefore, the welfare and health of Swiss dairy cows is of major interest. Many important epizootic pathogens, such as infectious bovine rhinotracheitis or brucellosis, have been eradicated or are being controlled to achieve a high health status. However, a known risk factor for transmission of pathogens is the commingling of animals from different herds (Gates et al., 2013). In Switzerland, approximately one-fifth of the cattle population traditionally spends the summers on alps (https://www.agrarbericht.ch/de/betrieb/strukturen/soemmerungsbetriebe, April 29, 2020) where animals from different farms graze together, increasing the risk of transmitting pathogens. It has for example been shown that the shared alpine pastures were an important risk factor for transmission of bovine viral diarrhoea virus in Switzerland (Braun et al., 1998). However, while we know about the importance of commingling animals for specific pathogens, such as bovine viral diarrhoea virus (Braun et al., 1998; Gates et al., 2013), the extent and dynamics of change it might induce in the whole microbiome is unknown.

Therefore, this study aims to get an overview of the composition of the nasal and faecal microbiome of Swiss dairy cows and the changes occurring upon mixing of herds. On the newly built AgroVet-Strickhof farm, a research and education facility for farming, three dairy herds were merged. This consolidation presented a unique opportunity to study the effects on the bovine microbiome when animals are intermingled. As part of an extended health examination upon re-location of the cows, nasal swabs, faecal samples and blood samples were taken of each animal before arriving in the in the new stable and approximately 6 months after the move to the new farm. NGS was conducted with nasal swabs and faecal specimen to observe the effects of mixing three independent dairy herds on their microbiome.

2 Materials and Methods

2.1 Farms and Animals

In 2017, a new research centre for agriculture and animal science was opened in Lindau ZH. The new facility is called AgroVet-Strickhof and is a cooperation of the Strickhof agricultural school, the ETH Zurich and the University of Zurich (UZH). Part of it is a newly built freestall barn for 120 dairy cows. In this new stable the research and education herds of the Strickhof, the ETH and the UZH were united to one herd. In this project the three research herds were sampled individually as well as the consolidated new AgroVet-Strickhof herd. Only animals of which we had two samples per sample type, meaning a pre and a post consolidation sample, were included in the study.

2.1.1 Strickhof herd (Strickhof, Lindau, ZH, Switzerland)

At the time of sampling the herd was housed in a freestall barn on a farm in Nürensdorf 3 km from AgroVet-Strickhof farm. Of this herd 61 nasal swabs, 60 faecal samples and 73 blood samples were obtained. For the nasal swab analysis 55 cows were included in the study, and for the faecal analysis 54 cows were included.

The majority of cows were younger than five years, and the breeds Braunvieh, Holstein and Red Holstein were equally represented *(Figure 1)*.

2.1.2 ETH herd (Chamau, Cham, ZG, Switzerland)

The research herd of the ETH was sampled between five and ten days after arrival on the AgroVet-Strickhof farm. This herd was the first to move into the newly built stable. The latter animals were sampled before the arrival of the other two herds at the AgroVet-Strickhof farm. Nasal swabs were taken from 51 animals and faecal samples from 44 cows. The blood was drawn from 52 animals upon leaving the farm in Chamau 50 km from AgroVet-Strickhof farm. For the analysis nasal swabs of 44 animals were included and faecal samples of 37 animals. Also in this herd the younger animals under five years were more frequent than older ones, and nearly 70% of the herd were the breed Holstein *(Figure 1)*.

2.1.3 UZH herd (Stigenhof, Oberembrach, ZH, Switzerland)

The research and veterinary teaching herd of the UZH was originally located at the Stigenhof in Oberembrach 6 km from the AgroVet-Strickhof farm. There, 15 animals were sampled for nasal swabs and faecal samples. From 22 animals blood samples was taken. The nasal swabs

and faecal samples of 11 cows were included in the study. In contrast to the other two farms, the proportion of animals over five years of age was here clearly higher than of younger animals. The breeds Red Holstein and Braunvieh were well represented, but in contrary to the other herds no cows belonged to the breed Holstein *(Figure 1)*.

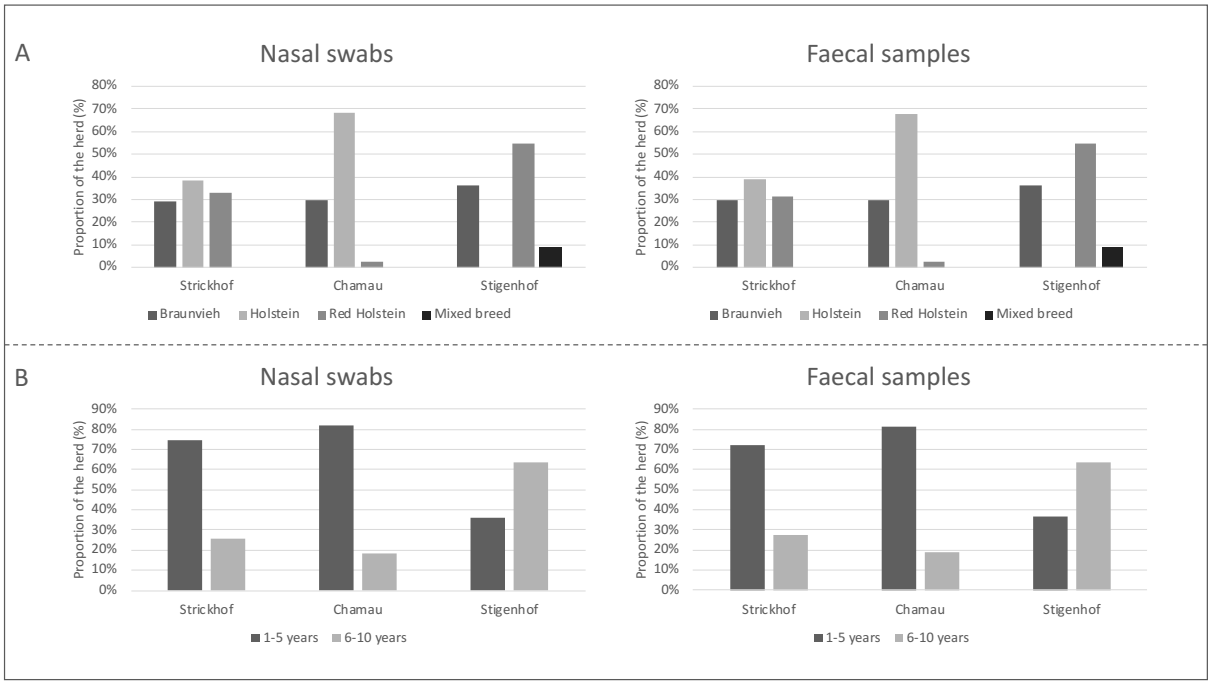


Figure 1: Characterization of the samples included in the study
Cattle breed (A) and age distribution (B) within the three herds (pre-sampling farms), Strickhof, Chamau and Stigenhof for the obtained nasal swabs and faecal samples.

2.1.4 AgroVet-Strickhof herd

From the newly consolidated herd 127 nasal swabs, 126 faecal samples and 126 blood samples were taken. Out of these, only 110 nasal swabs and 102 faecal samples were included in the study because only these animals were also sampled before the consolidation of the herds. *(Figure 2)*

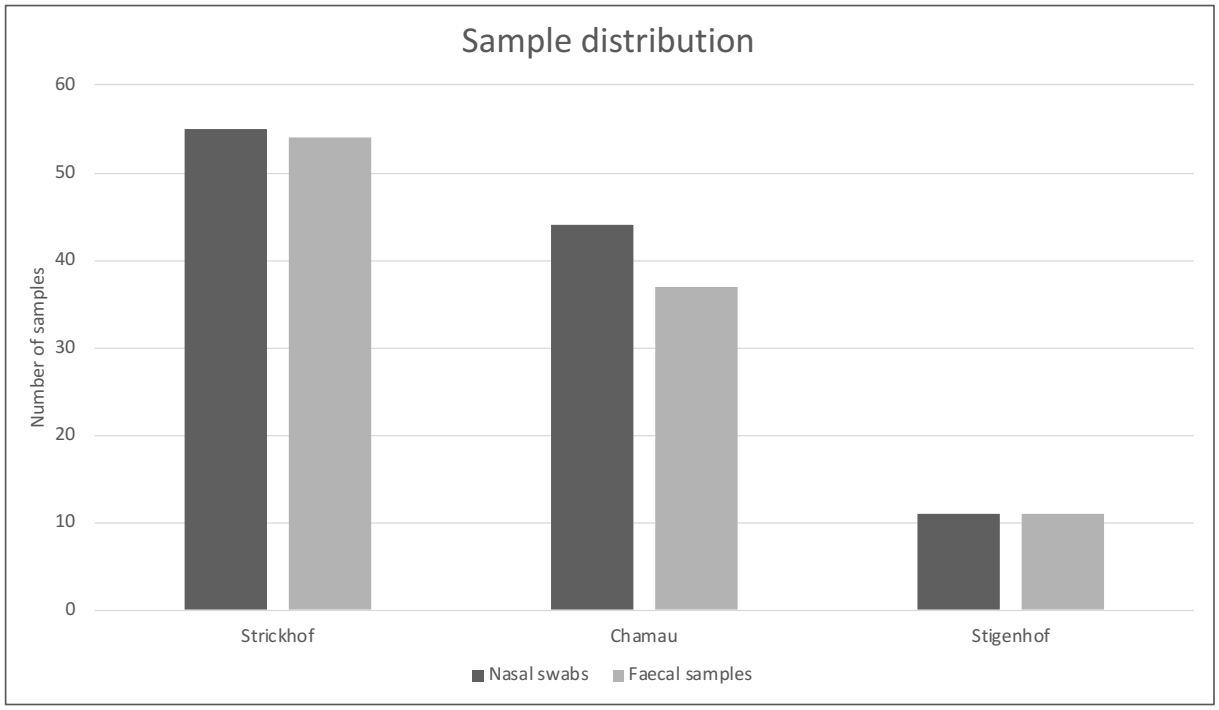


Figure 2: Sample distribution
Number of samples from the different herds (pre-sampling farms) included in the study.

2.2 Sample collection

For this project the animals of three dairy cow herds were sampled. Of each animal two nasal swabs, two faecal samples and two blood samples were taken, one prior and one after consolidation.

After taking the nasal swabs (Flexible Minitip Flocked Swab 100mm, 503CS01, Copan Diagnostics, Murrieta CA, USA) they were placed individually in a sterile 15ml plastic tube and 1ml of 1x phosphate-buffered saline (PBS) was added. They were then stored at -20°C until testing.

Faecal samples were taken rectally with the aid of glycerol. Faeces was added to a sterile plastic container and stored at -20°C until testing.

From the caudal vein 10ml EDTA blood was taken. The blood samples were centrifuged at 868xg for 10 minutes to separate the plasma from the cellular blood fraction. The plasma fraction was poured off and stored in a sterile tube at -20°C. The volume x 4 of lysis buffer (0.15M NaH_4Cl, 10mM CHKO3, 0.1mM EDTA disodium salt [pH 7.2]) was added to 1x the volume of cellular blood fraction. After centrifugation at 2000xg for 10 minutes the supernatant was poured off. The leucocyte pellet was subsequently resuspended in 1x the volume of lysis buffer and again centrifuged for 10 minutes at 2000xg. The supernatant was again poured off, and this was repeated until there was only a white leucocyte pellet left. In the end the pellet was resuspended in 1ml of PBS and centrifuged at 3500g for 2 minutes. The supernatant was poured

off and the pellet stored at -20°C. The blood samples were taken for possible serological or virologic follow-up analyses but were not used during the course of this thesis.

2.3 Sampling dates

The nasal swabs and faecal samples from before consolidation where taken on three different days in March and April 2017. Each herd was sampled on a different farm. The first blood sample of each cow was taken upon moving to the AgroVet-Strickhof farm between February and May 2017. The samples after consolidation were taken on two different dates, approximately half a year and one year after the first samples were taken. Most of the cows were re-sampled in October 2017. The cows not present at the AgroVet-Strickhof farm at that day were then re-sampled in February 2018. *(Table 1)*

Herd	Nasal swab-pre	Nasal swab-post	Faeces-pre	Faeces-post	Blood-pre	Blood-post
Strickhof	April 2017	October 2017/ February 2018	April 2017	October 2017/ February 2018	February/ May 2017	October 2017/ February 2018
Chamau (ETH)	March 2017	October 2017/ February 2018	March 2017	October 2017/ February 2018	February 2017	October 2017/ February 2018
Stigenhof (UZH)	April 2017	October 2017/ February 2018	April 2017	October 2017/ February 2018	April 2017	October 2017/ February 2018

Table 1: Sampling dates
The table shows the sampling time points of the three herds (pre-sampling farms) and different sample types: "pre" samples were taken before the moving of the herds to the AgroVet-Strickhof farm, "post" samples were taken of the animals after the consolidation.

2.4 Sample pooling

A total of 424 samples were included in this study. Since analysing them all individually would have exceeded the temporal and financial limits of this study, the samples of up to six animals of similar age and with similar origin and movement histories were pooled together. The movement history of each sampled cow was examined by consulting the Swiss Animal Tracing Database in which the whereabouts of a cow must be registered at any given timepoint. The duration of the stay of an individual cow on each farm was calculated. The farm a cow stayed the longest on was considered the main farm. In a first step, all animals were grouped according to their main farm. As the maximum animals per pool were six individuals, further pooling criteria were applied if the number of animals per main farm exceeded six. The further pooling criteria were farm of birth, alpine summer farm and further farms the cow lived on. The following main farms were identified: Chamau, Levy, Münchwilen, Nürensdorf, Stigenhof,

Strickhof. Additionally, eight main farms with only one pool of animals were identified: Collenberg Morissen, Fankhauser Emmenmatt, Jungen-Minnig, Manetsch, Richner Unterkulm, Ender-Meier, Brunner Waltensburg, Caduff. These farms were summarized as "übrige Betriebe".

After applying these criteria to the samples, 36 nasal swab pools and 34 faecal pools were created *(Table 2)*. For the nasal swabs two pools per group were made, one for the DNA extraction and one for the RNA extraction. For the DNA extraction each sample was individually extracted, and a total volume of 36μl extracted nucleic acid was mixed together per pool. For the RNA extraction a final pool volume of 600μl nasal swab fluid was blended.

For the faecal pooling the samples of each group were thawed and pooled together in an Eppendorf tube to a total end weight of 1020mg faeces per pool.

The pool end-volume, respectively end-weight, was chosen in order to supply enough material to perform sequencing runs for bacteria and viruses from the same pool mix.

A

Nasal swabs	Pool name	Number of animals	Main farm	Pre-sampling farm
	01N	1	Chamau	Chamau
	02N	4	Chamau	Chamau
	03N	3	Chamau	Chamau
	04N	6	Chamau	Chamau
	05N	3	Chamau	Chamau
	06N	5	Chamau	Chamau
	07N	6	Chamau	Chamau
	08N	6	Chamau	Chamau
	09N	6	Chamau	Chamau
	10N	1	Collenberg Morissen	Stigenhof
	11N	1	Fankhauser Emmenmatt	Chamau
	12N	1	Jungen-Minnig	Chamau
	13N	4	Levy	Strickhof
	14N	3	Levy	Strickhof
	15N	2	Levy	Strickhof
	16N	6	Manetsch	Strickhof
	17N	6	Münchwilen	Strickhof
	18N	5	Münchwilen	Strickhof
	19N	3	Münchwilen	Strickhof
	20N	3	Münchwilen	Strickhof
	21N	3	Nürensdorf	Strickhof
	22N	2	Nürensdorf	Strickhof
	23N	1	Richner Unterkulm	Chamau
	24N	5	Stigenhof	Stigenhof
	25N	1	Stigenhof	Stigenhof
	26N	2	Stigenhof	Stigenhof
	27N	1	Stigenhof	Stigenhof
	28N	4	Strickhof Lindau	Strickhof
	29N	4	Strickhof Lindau	Strickhof
	30N	1	Strickhof Lindau	Strickhof
	31N	1	Strickhof Lindau	Strickhof
	32N	4	Strickhof Lindau	Strickhof
	33N	2	Strickhof Lindau	Strickhof
	34N	1	Ender-Meier	Chamau
	35N	2	Brunner Waltensburg	Strickhof
	36N	1	Caduff	Strickhof

B

Faecal samples	Pool name	Number of animals	Main farm	Pre-sampling farm
	01F	4	Chamau	Chamau
	02F	3	Chamau	Chamau
	03F	5	Chamau	Chamau
	04F	3	Chamau	Chamau
	05F	6	Chamau	Chamau
	06F	6	Chamau	Chamau
	07F	6	Chamau	Chamau
	08F	1	Collenberg Morissen	Stigenhof
	09F	1	Fankhauser Emmenmatt	Chamau
	10F	1	Jungen-Minnig	Chamau
	11F	4	Levy	Strickhof
	12F	3	Levy	Strickhof
	13F	1	Levy	Strickhof
	14F	6	Manetsch	Strickhof
	15F	6	Münchwilen	Strickhof
	16F	4	Münchwilen	Strickhof
	17F	3	Münchwilen	Strickhof
	18F	3	Münchwilen	Strickhof
	19F	3	Nürensdorf	Strickhof
	20F	2	Nürensdorf	Strickhof
	21F	1	Richner Unterkulm	Chamau
	22F	5	Stigenhof	Stigenhof
	23F	1	Stigenhof	Stigenhof
	24F	2	Stigenhof	Stigenhof
	25F	1	Stigenhof	Stigenhof
	26F	5	Strickhof Lindau	Strickhof
	27F	4	Strickhof Lindau	Strickhof
	28F	1	Strickhof Lindau	Strickhof
	29F	1	Strickhof Lindau	Strickhof
	30F	4	Strickhof Lindau	Strickhof
	31F	2	Strickhof Lindau	Strickhof
	32F	1	Ender-Meier	Chamau
	33F	2	Brunner Waltensburg	Strickhof
	34F	1	Caduff	Strickhof

Table 2: Overview of the sample pools
Nasal swab pools (A) and faecal pools (B) with the corresponding number of animals per pool, the main farm and the pre-sampling farm. The main farm is the farm the animals spent the longest time on, the pre-sampling farm corresponds to the research herd the animal belonged to during pre-sampling.

2.5 Sample preparation

2.5.1 16S rRNA sequencing

For the determination of the bacteriome in the nasal swabs and faecal samples 16S rRNA sequencing was performed. For the phylogenetic classification the amplicon of the V3 and V4 region of the 16S rRNA was sequenced. For all centrifugation steps a microcentrifuge (Biofuge

pico, Heraeus, Hanau, Germany) was used. If the sample preparation continued the next day the samples were stored at 4°C overnight, otherwise they were frozen at -20°C until further used.

2.5.1.1 Nucleic acid extraction

To extract the nucleic acid (NA) for the 16S rRNA sequencing two different extraction kits were used. For extraction of the NA of the nasal swabs the QIAmp DNA Mini Kit (QIAGEN AG, Hombrechtikon, Switzerland) was used according to the manufacturer's protocol. As starting material 400μl of nasal swab fluid was used, and the NA was eluted in 100μl of nuclease-free water. For the extraction of the NA out of the faecal samples the QIAmp DNA Stool Mini Kit (QIAGEN AG, Hombrechtikon, Switzerland) was used according to the manufacturer's protocol. Of each sample pool 200mg of faeces was used for the extraction, and the NA was eluted in 200μl of buffer AE.

2.5.1.2 Amplification Polymerase Chain Reaction

For the amplification polymerase chain reaction (PCR) of the V3 and V4 region the primers 16S_341F and 16S_805R (Herlemann et al., 2011) with Illumina adapter sequences added were used *(Table 3)*. During the mixture preparation all samples were kept on ice. For this PCR the reaction mixture contained 0.2mM of each deoxynucleotide triphosphate (dNTP), 0.5μM of the forward and 0.5μM of the reverse primer, 400ng/μl of bovine serum albumin (BSA), 1x Phusion HF buffer and 0.02U/μl of Phusion Hot Start II Hight Fidelity Polymerase (Thermo Fisher Scientific, Waltham, MA USA). Of the template DNA 5μl were added to the reaction mixture and then topped up with nuclease-free water to a total reaction volume of 50μl. The following cycling conditions were applied: A 30 second activation step at 98°C, followed by 35 cycles of 10 seconds at 98°C, 30 seconds at 54°C and 30 seconds at 72°C. For the final extension the samples were incubated for 10 minutes at 72°C. The PCR reactions were carried out on a MJ Research PTC-200 Peltier Thermal Cycler. After the amplification 5μl of DNA Gel Loading Dye (6x) (Thermo Fisher Scientific, Waltham, MA USA) was added to each sample and then loaded onto a 1.5% Agarose Gel (Agarose, LE, Analytical Grade; Promega, Dübendorf, Switzerland) containing 6x Gel Red 10,000 in water (Biotium, Fremont CA, USS). The Gene Ruler 100bp DNA ladder was used as a marker. The gel electrophoresis was run at 100V for approximately 60 minutes. The bands of the PCR product were made visible under UV light, and the band of the expected length of 530 bp was cut out with a sterile scalpel blade. Gel

extraction was performed with the QIAquick Gel Extraction Kit (QIAGEN AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol.

Forward primer	**Illumina adapter:**	**Random hexamer**	**V3/4 forward primer 341f:**
	5'-CTTTCCCTACACGACGCTCTTCCGATCT	NNNNNNNN	CCTACGGGNGGCWGCAG-3'
Reverse primer	**Illumina adapter:**	**Random hexamer**	**V3/4 reverse primer 805r :**
	5'-GGAGTTCAGACGTGTGCTCTTCCGATCT	NNNNNNNN	GACTACHVGGGTATCTAATCC-3'

Table 3: Primers used for the 16S rRNA amplification PCR
Sequences of the specific primers for the V3/V4 region of the prokaryotic 16S rRNA genome with Illumina adapters attached.

2.5.1.3 Index PCR

In a second PCR step, indices were added to the amplification product to allow multiplexed sequencing. The NEBNext i501 Primer – i508 Primer and NEBNext i701 Primer – i712 Primer (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), New England Biolabs, Ipswich MA, USA) were used to individually label the samples *(Table 4)*. The reaction mixture was kept on ice. For each sample a unique i5/i7 primer pair was used. The reaction mixture contained 0.2mM of each dNTP, 0.5μM of an i5 and 0.5μM of an i7 primer, 1x Phusion HF buffer and 0.02U/μl of Phusion Hot Start II Hight Fidelity Polymerase (Thermo Fisher Scientific, Waltham, MA USA). To the reaction mixture 15μl of template DNA from the amplification PCR was added, and then the reaction volume was topped up with nuclease-free water to 50μl. The PCR condition included 30 seconds of activation at 98°C, followed by 20 cycles of 10 seconds denaturation at 98°C, 30 seconds at 54°C for annealing and 30 seconds at 72°C for the extension. For the final extension the samples were incubated for 10 minutes at 72°C. The PCR reactions were carried out on a MJ Research PTC-200 Peltier Thermal Cycler. After the amplification 5μl of DNA Gel Loading Dye (6x) (Thermo Fisher Scientific, Waltham, MA USA) was added to each sample and then loaded onto a 1.5% Agarose Gel (Agarose, LE, Analytical Grade; Promega, Dübendorf, Switzerland) containing 6x Gel Red 10,000 in water (Biotium, Fremont CA, USS). The Gene Ruler 100bp DNA ladder was used as a marker. The gel electrophoresis was run at 100V for approximately 60 minutes. The bands of the PCR product were made visible under UV light and the band of the expected length of 600 bp was consequently cut out with a sterile scalpel blade. Gel extraction was done with the QIAquick Gel Extraction Kit (QIAGEN AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol.

Primer 5	Index i5	Illumina adapter
5'-AATGATACGGCGACCACCGAGATCTACAC	(i5)	ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'
Primer 7	**Index i7**	**Illumina adapter**
5'-CAAGCAGAAGACGGCATACGAGAT	(i7)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T-3

Table 4: PCR primers used for the 16S rRNA index PCR
For each sample a unique i5/i7 primer pair was used to later distinguish the sequenced reads.

2.5.1.4 Bacteriome Sequencing

The concentration of the amplicon libraries was checked on the TapeStation 2200 according to the manufacturer's protocol (Agilent Technologies, Santa Clara CA, USA). Depending on concentration of the sample, the Hight Sensitivity D1000 or the D1000 screen assay was used. Each sample was diluted to 4nM. Of each nasal swab 5μl of the library was added to a new tube to form one pool of nasal swab samples, and the same was done for the faecal samples. The nasal swab library pool and the faecal sample library pool were sequenced in two separate runs. The DNA concentration of each library pool was checked on the TapeStation 2200 according to the manufacturer's protocol (Agilent Technologies, Santa Clara CA, USA) and diluted to 2nM. To make sure that it was indeed 2nM, the final DNA concentration of the pool was controlled again on the TapeStation 2200 according to the manufacturer's protocol (Agilent Technologies, Santa Clara CA, USA). Protocol A of the MiSeq System Denature and Dilute Libraries Guide (Illumina, San Diego CA, USA) was used to prepare the library pools for sequencing. As a control 35% of PhiX was added, and the library pools were subsequently sequenced on the Illumina MiSeq (Illumina, San Diego CA, USA) using the MiSeq Reagent Kit v3. The samples were sequenced paired end 2x 300 bp.

2.5.2 Virome protocol

To characterize the nasal and faecal virome, an inhouse sample preparation protocol was used. The aim of this protocol is a relative enrichment of viral particles within the sample. For all centrifugation steps a microcentrifuge (Biofuge pico, Heraeus, Hanau, Germany) was used. If the samples were further used the next day, they were stored at 4°C overnight, otherwise they were frozen at -20°C until further used.

The virome of the 36 nasal swab pools and 34 faecal pools was sequenced. Additionally, four animals were individually sequenced. The two nasal swabs and two faecal samples of a twin pair of cows to see if the virome of twins is more similar than that of other cows. In order to analyse the effect of pooling on sensitivity of virus detection, samples of two cows were sequenced pooled as well as individually.

2.5.2.1 Enrichment

Since viruses have no common genomic feature, such as 16S in bacteria, untargeted shotgun sequencing was used for virome analysis. With this approach, all nucleic acids present in a sample are sequenced, also the unwanted host RNA and DNA. In order to increase the proportion of viral nucleic acid a relative enrichment step, to remove free RNA and DNA unprotected by a viral capsid, was performed.

The enrichment protocol consists of three different steps: homogenisation, filtration and nuclease treatment. Of each nasal swab pool 300μl was homogenized in a 2ml Eppendorf tube for 1 minute at 20 Hz in a TissueLyzer II (QIAGEN AG, Hombrechtikon, Switzerland). The homogenized samples were then centrifuged at full speed for 5 minutes. The supernatant was aspirated using a 1ml syringe and then pushed through a syringe filter (13mm Disposable Filter Device, Whatman, GE Healthcare Life Sciences, Glattbrugg, Switzerland) with a pore size of 0.45μm. In a 0.2ml PCR tube the following reagents were mixed together: 134μl of homogenized and filtrated sample, 14μl of Micrococcal Nuclease Buffer, 1μl of Micrococcal Nuclease (New England Biolabs, Ipswich MA, USA) and 1μl of RNase A 20-40mg/ml (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) or 1μl RNase A 10mg/ml (Thermo Fisher Scientific, Waltham, MA USA). The reaction mixture was incubated for 15 minutes at 45°C and then for 1 hour at 37°C.

2.5.2.2 Nucleic acid extraction

For nucleic acid (NA) extraction the QIAmp Viral RNA Mini Kit (QIAGEN AG, Hombrechtikon, Switzerland) was used. Slight modifications to the manufacturer's protocol were made, such as omitting the carrier RNA and adding β-Mercaptoethanol. In a first step, β-Mercaptoethanol and Buffer AVL were mixed in a ratio 1:100. Of this mixture 600μl was added to 150μl of each sample. The β-Mercaptoethanol is needed to inactivate the nucleases from the previous step. Further, 600μl instead of 560μl of 100% ethanol per samples was used. A two-step elution was conducted using 20μl of nuclease-free water and subsequently 20μl of Tris-EDTA buffer.

2.5.2.3 Reverse transcription and second strand synthesis

In this protocol sequence-independent, single-primer amplification (SISPA), a method for unspecific amplification of nucleic acid in a sample (Reyes and Kim, 1991), was used in order to get a sufficient DNA concentration for subsequent library preparation. It is based on

introducing a known genetic tag anchored to random hexamers into the cDNA and second strand DNA that can subsequently be used as primer binding site for amplification.

For the following steps all samples were kept on ice. For the reverse transcription two reaction mixtures were prepared: i) The cDNA-mix, containing 1x Reaction Buffer, 1nM of dNTP mix, 1U/μl of RiboLock RNase Inhibitor and 10U/μl of RevertAid H Minus M-MuLV Reverse Transcriptase (RevertAid H Minus First Strand cDNA Synthesis Kit, Thermo Fisher Scientific, Waltham, MA USA) and ii) The Reserve Transcription (RT) pre-mix, containing 28.75μl of sample and 2.5μM of SISPA-N primer *(Table 5)*. The Reserve Transcription (RT) pre-mix was incubated at 97°C for 3 minutes and subsequently placed on ice for at least 1 minute. After cooling down, 20μl of cDNA-mix was added and mixed well. The reaction mixture was then incubated for 10 minutes at 25°C to facilitate primer binding, followed by incubation at 42°C for 90 minutes and 70°C for 5 minutes and then placed on ice. After cooling, 1μl of RNase H 5U/μl (New England Biolabs, Ipswich MA, USA) was added to each sample and incubated at 37°C for 20 minutes. For the second strand synthesis 0.4μl of 100μM SISPA-N primer, 0.6μl of 10x Klenow buffer (Klenow Fragment, exo- (5U/μl), Thermo Fisher Scientific, Waltham, MA USA) and 1μl of 10mM dNTP mix was added to the cDNA of each sample. The mixture was then denatured at 95°C for 1 minute and immediately cooled on ice. Next, 2.5μl of Klenow Fragment, exo- (5U/μl) (Thermo Fisher Scientific, Waltham, MA USA) was added to each sample and incubated at 25°C for 15 minutes, afterwards at 37°C for 1 hour. Subsequently, the samples were again denatured at 95°C for 1 minute and immediately stored on ice. Again, 1.25μl of Klenow Fragment, exo- (5U/μl) (Thermo Fisher Scientific, Waltham, MA USA) was added and incubated at 25°C for 15 minutes, followed by 1 hour at 37°C for a second round of second strand synthesis. The samples were then purified using the PureLink PCR Micro Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA USA) according to the manufacturer's protocol, with exception of the elution step where 12μl of Elution Buffer was used instead of 10μl to ensure enough purified DNA for the following steps.

Primer	**Sequence**
SISPA-N	GTTGGAGCTCTGCAGTCATCNNNNNN
SISPA	GTTGGAGCTCTGCAGTCATC

Table 5: SISPA primers
Sequences of sequence-independent, single-primer amplification (SISPA) primers for the random amplification step.

2.5.2.4 Amplification

The reaction mix for the amplification PCR contained 1x 10x PCR Buffer, 0.2mM of dNTP mix, 0.8μM of SISPA primer *(Table 5)* and 0.05 U/μl HotStarTaq DNA Polymerase (QIAGEN

AG, Hombrechtikon, Switzerland). Of the template DNA 10μl were added, and nuclease-free water was filled up to a reaction volume of 50μl. The following PCR conditions were used: A 15 minutes activation step at 95°C, followed by 20 cycles of 30 seconds of denaturation at 94°C, 30 seconds annealing at 58°C and 1 minute of extension at 72°C. The final extension was performed at 72°C within 10 minutes. The amplified samples were then purified using the QIAquick PCR Purification Kit (QIAGEN AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol using 30μl of elution buffer. Subsequently, the DNA concentration of each sample was measured by a Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA USA) using the Qubit 1x dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA USA) according to the manufacturer's protocol.

2.5.2.5 Library preparation

For the library preparation 1ng DNA of each nasal swab and 3ng DNA of each faecal sample were pipetted into new tubes and the volume topped up to 50μl with elution buffer. The samples were then acoustically sheared to 500bp with the focused-ultrasonicator Covaris E220 (Covaris Inc., Woburn MA, USA). The libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit for Illumina according to the manual. For the nasal swabs 9 amplification cycles were used, for the faecal samples 8. For purification AMPure XP magnetic beads (Beckman Coulter Life Sciences, Inidianapolis IN, USA) were used.

2.5.2.6 Virome Sequencing

The concentration and fragment size distribution of the libraries was measured on the TapeStation 2200 (Agilent Technologies, Santa Clara CA, USA) using the High Sensitivity D1000 or D1000 screen assay, depending on the library concentration. The nasal libraries were then diluted to 1nM and the faecal libraries to 2nM. Two final pools were mixed, one containing 5μl of each diluted nasal library and another containing 5μl of each faecal library. The final pools were again checked for the correct concentration on the TapeStation. Subsequently, the two pools were denatured and 1% PhiX library was added according to the manufacturer's instruction manual. The pools were then sequenced on an Ilumina NextSeq 550 (QIAGEN AG, Hombrechtikon, Switzerland), paired end 2x 150 base pairs, in two separate high output runs.

2.6 Data analysis

2.6.1 16S rRNA MiSeq data

The 16S rRNA sequencing data was analysed by MWSchmid GmbH (http://mwschmid.ch/). Operational taxonomic units (OTUs) were generated with UPARSE (usearch version 10.0.024, (Edgar, 2013)) following the example and the tutorial given for paired-end Illumina data (http://drive5.com/uparse/). Reads were first quality-checked with FastQC (bioinformatics.babraham.ac.uk/projects/fastqc). Following removal of sequencing adapters and low-quality bases with Trimmomatic (version 0.36 with the parameters ILLUMINACLIP:adapterSeqs:2:30:10 SLIDINGWINDOW:5:15 MINLEN:100, (Bolger et al., 2014)), paired-end reads were merged and then filtered using usearch (with the parameters -fastq_maxdiffs 25 for merging and -fastq_maxee 1 for filtering, (Edgar, 2013)). Merged reads were then truncated up to the 16S primer sequences (forward: CCTACGGGNGGCWGCAG, reverse: GACTACHVGGGTATCTAATCC) and filtered for the presence of both primer sequences with a custom python script allowing up to 2 mismatches per primer. Duplicated sequences were then collapsed with usearch, and the resulting unique sequences were clustered with usearch (99% identity and with the parameter -minsize 2, (Edgar, 2013)) to obtain 1,390 nasal OTU and 1,344 faecal OTU sequences. OTU sequences were annotated with the taxonomy data using the RDP 16S training set (version 16, http://drive5.com/sintax/rdp_16s_v16_sp.fa.gz) and the usearch -sintax command with the parameter -sintax_cutoff set to 0.8. OTU abundances were finally obtained by counting the number of sequences (merged and filtered) matching to the OTU sequences (usearch -otutab command with default parameters, (Edgar, 2013)). OTUs annotated as chloroplast were removed to avoid a potential bias caused by plant DNA. To avoid sequencing artifacts, OTU sequences with less than 5 counts in any of the samples were removed from all further analyses (1,009 nasal and 965 faecal OTUs remained after this filter).

Normalized OTU counts were calculated accordingly with DESeq2 and log2(x+1)-transformed to obtain the normalized OTU abundances.

The medoid, a representative sample composition, of a group of samples was extracted using the function pam() ("Partitioning Around Medoids") from the library "cluster" in R (Maechler et al., 2019) by calculating a single cluster and extracting the medoid.

Differences in OTU relative abundance between groups before and after the consolidation were analysed with a generalized linear model in R with the package DESeq2 (version 1.14.1, (Love et al., 2014)) according to a design with a factor for the group (GROUP) and a factor for the sampling time-point (SAMPLING). The formula was ABUNDANCE ~ GROUP +

SAMPLING. *P*-values were adjusted for multiple testing (Benjamini and Hochberg, 1995), and OTUs with an adjusted *P*-value (false discovery rate, FDR) below 0.01 and a minimal log2 fold-change (i.e., the difference between the log2 transformed, normalized OTU counts) of 0 were considered to be differentially abundant.

Samples were clustered into k = 2, 3, 4, or 5 groups using the "Partitioning Around Medoids" algorithm (function pam() in R, (Reynolds et al., 2006)). We then searched for variables that were associated with the clustering with Boruta (Kursa and Rudnicki, 2010).

To test for enrichment/depletion of bacterial taxa occurrences in a given set of OTUs (e.g., OTUs with significant difference in abundance between the two sampling time points), we constructed for each taxum a contingency table with the within/outside taxum counts for the given set of OTUs and all OTUs passing the filter. We then tested for significance with Fisher's exact test. *P*-values were adjusted for multiple testing (Benjamini and Hochberg, 1995), and phyla with an adjusted *P*-value (false discovery rate, FDR) below 0.05 were considered to be significantly enriched/depleted.

The sample specificity was calculated based on the tissue specificity index (Yanai et al., 2005).

To characterize the overall impact of the consolidation of the three pre-sampling herds on the bacterial community structure, we analysed the variation in OTU richness, diversity (Shannon Index), effective richness (exponent of the Shannon index, (Magurran, 2004) and evenness (Pielou, 1975) between the groups before and after the consolidation. The formula was INDEX ~ GROUP + SAMPLING.

To assess the overall impact of the consolidation of the three pre-sampling herds on the dissimilarity between the microbiomes' bacterial community structure, we analysed the variation in dissimilarities between microbiomes with a multivariate ANOVA in R with the package vegan (version 2.4-4, function adonis(); (Oksanen et al., 2017)). We used the Manhattan distance as a dissimilarity measure because it has been shown to be consistently more preferable than the Euclidean distance for high dimensional data (Aggarwal et al., 2001). The formula was DISTANCE ~ GROUP + SAMPLING.

To visualize the changes in the overall bacterial community structure (i.e., Manhattan distances), we calculated the pairwise distance between groups of each main farm before and after the consolidation of the herds (Webb et al., 2002).

2.6.2 Virome NextSeq data

The reads of the two NextSeq runs were analysed with the web-based Sushi platform provided by the Functional Genomics Center Zurich (https://fgcz-sushi.uzh.ch). The raw nasal and faecal

reads were run on the VirDetect application implemented there. Raw reads were pre-processed using Trimmomatic (parameters: 5 prime hard trimmed: 5 bp; minTailQuality: 10; minAvgQuality: 20; minReadLength: 50) to remove adaptors and low-quality regions. Trimmed and filtered reads were mapped to the Human genome (Ensembl GRCh38.p10) to remove contamination introduced during human handling using bowtie2 (parameters: very-sensitive). Unmapped reads were extracted using samtools and aligned to the host genome (Ensembl Cow UMD 3.1) to remove host contamination using bowtie2 with the same parameter setting. Unmapped reads were extracted again and aligned to the custom made virome database with 55,918 full viral genomes using bowtie2 (parameters: -a --very-sensitive --no-mixed --no-discordant -X 1000). Mapped reads and mapped bases per viral genome were calculated using bedtools. In nasal swabs viral genomes with at least 1 mapped read and in faecal samples viral genomes with at least 10 mapped reads were reported using R markdown (http://rmarkdown.rstudio.com/). For further analysis only viral genomes with 1% coverage or more were considered. Phages and known plant related viruses were excluded.

To get access to the read sequences and to check specifically and with higher sensitivity for viruses of interest, such as bovine related viruses, all samples were additionally screened using the SeqMan NGen, Lasergene software version 16 (DNASTAR, Madison WI, USA) to purposely made sequence databases, e.g., containing only viruses known to infect cattle, compiled from GenBank. The contigs were visualized and analysed on the SeqMan Pro, Lasergene software version 16 (DNASTAR, Madison WI, USA).

For the statistical analysis IBM SPSS Statistics (version 25) was used. For quantitative analysis Wilcoxon signed rank tests were conducted, for qualitative analysis the McNemar test was used.

3 Results

3.1 Bacteriome

72 nasal swab samples were sequenced with a mean number of 267,952.5 raw reads per sample and 68 faecal samples with a mean number of 298,721.5 raw reads per sample. After data processing 1,009 OTUs in nasal swab samples and 965 OTUs in faecal samples were used for further analysis. The analysis and calculations of the bacteriome were conducted by MWSchmid GmbH.

3.1.1 Representative samples

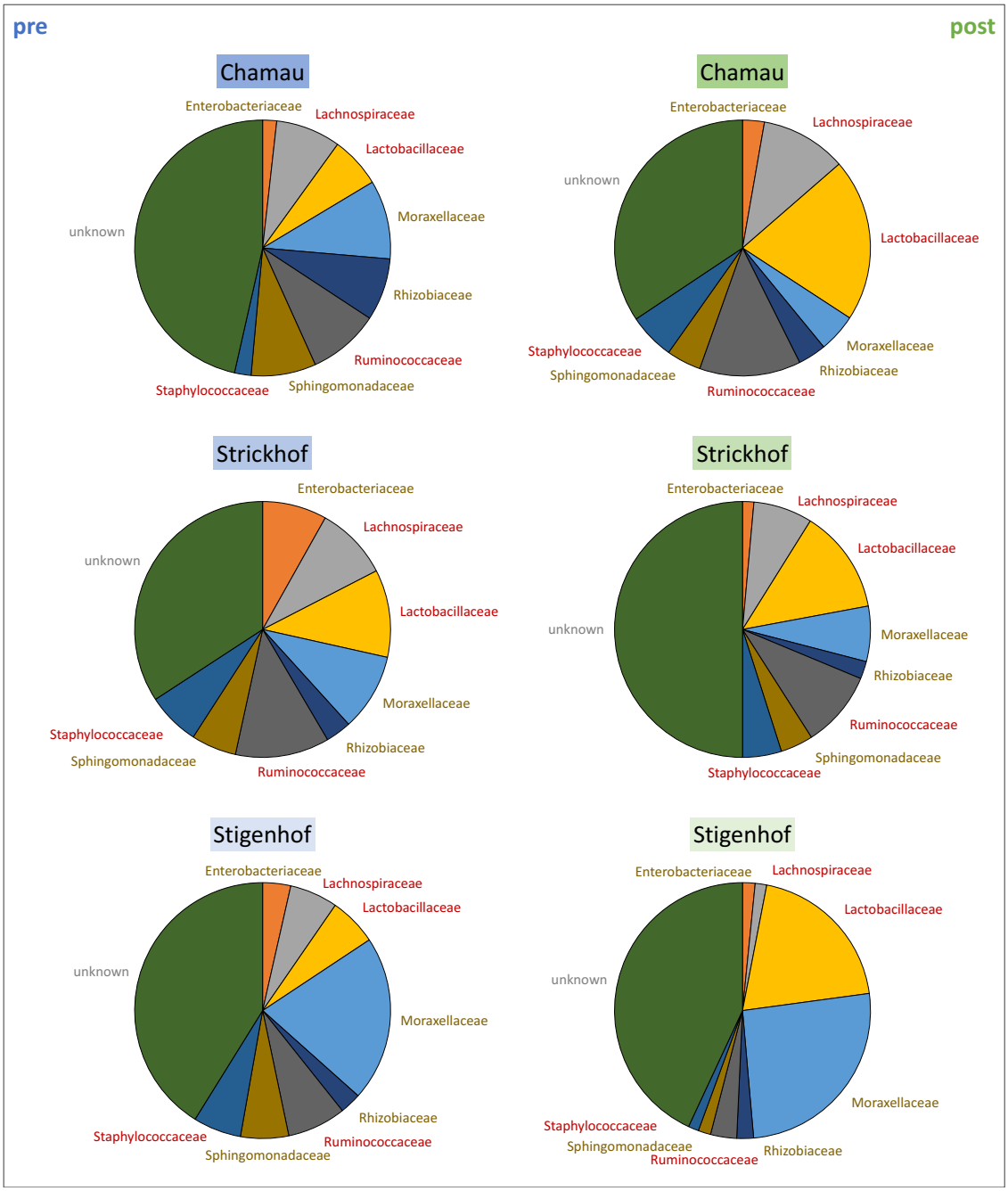
All analyses were performed with normalized reads. Normalization was computed with DESeq2 as described in chapter *2.6.1*. The sequences were normalized to the expected expression of the respective sequence. Summing up the reads over all samples, four phyla (*Proteobacteria*, 37.01%; *Firmicutes*, 32.48%; *Bacteroidetes*, 15.71%; *Actinobacteria*, 11.9%) accounted for 97.1% of the reads in nasal swabs. In faecal samples two phyla (*Firmicutes*, 61.87%; *Bacteroidetes*, 34.56%) accounted for 96.43% of the reads. In order to identify the representative bacteriome composition, the medoid of bacterial families was calculated for each pre-sampling farm *(Figure 3)*. The medoid is a sample composition that displays minimum distance between members of a dataset and therefore can be considered a representative member of a group.

In the nasal swabs of all pre-sampling farms between half and a third of the bacterial reads could not be taxonomically assigned on the family level. On all three farms the same eight bacterial families were identified. In the pre-sampling farm "Chamau" *Moraxellaceae* were the most frequent bacteria in the pre samples and *Lactobacillaceae* in the post samples. In the nasal swabs of the pre-sampling farm "Strickhof" *Ruminococcaceae* was the most common bacterial family in the pre samples and *Lactobacillaceae*, as in the samples from "Chamau", the most frequently detected family in the post samples. In the pre and post samples of the pre-sampling farm "Stigenhof" the most prevalent family was *Moraxellaceae*, although more frequent in the post medoid sample.

In the faecal samples of the pre-sampling farms "Chamau", "Strickhof" and "Stigenhof" between 53% and 56% of the reads could not be assigned to a bacterial family. The same six bacterial families were present in all faecal medoid samples of which *Ruminococcaceae* were the most common in every medoid sample. The composition of all faecal medoid samples is much more similar among each other than the nasal medoid samples among themselves.

Two bacterial families, *Lachnospiraceae* and *Ruminococcaceae*, were found in all nasal swab and faecal medoid samples, pre and post, of all three pre-sampling farms.

A



B

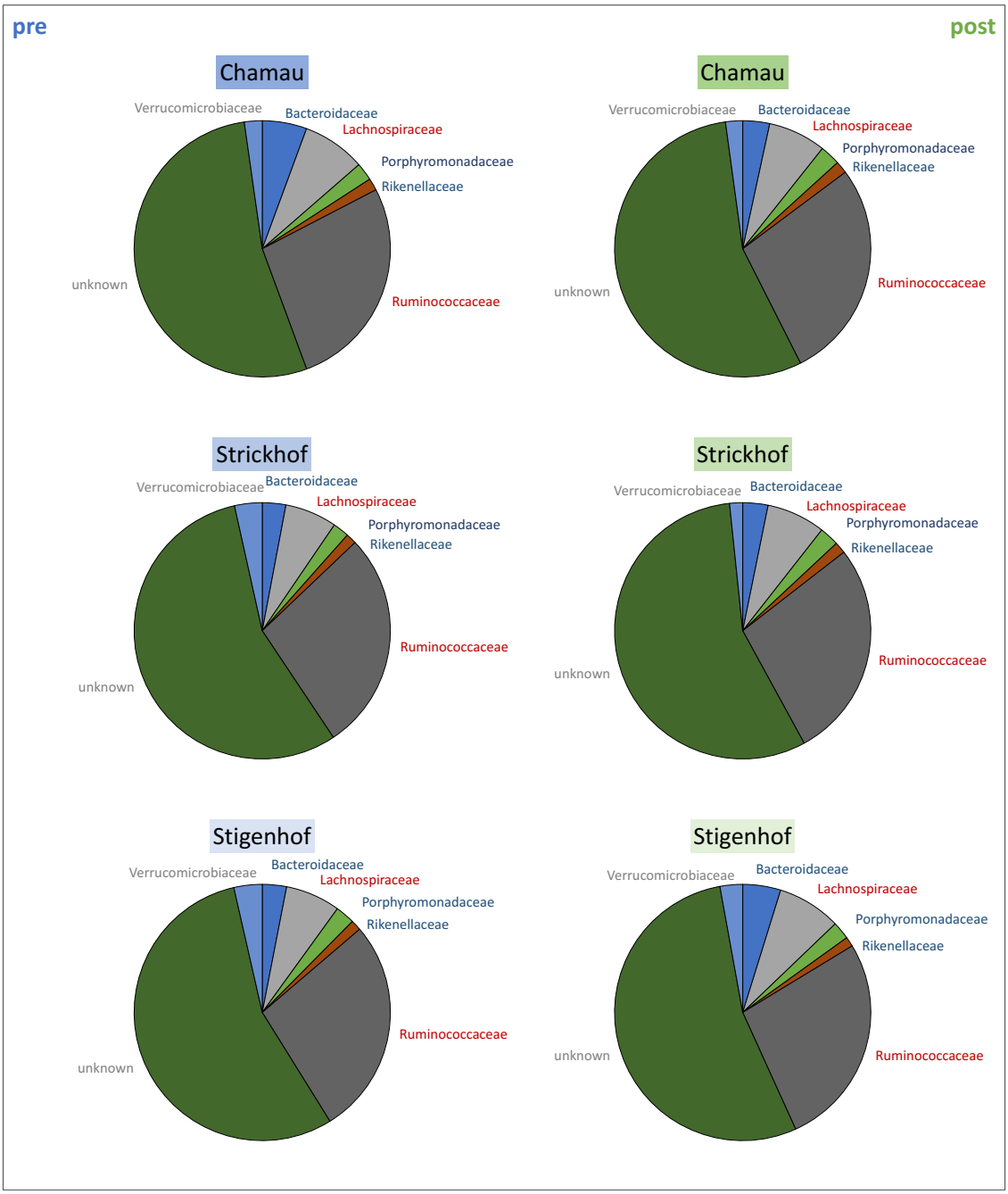
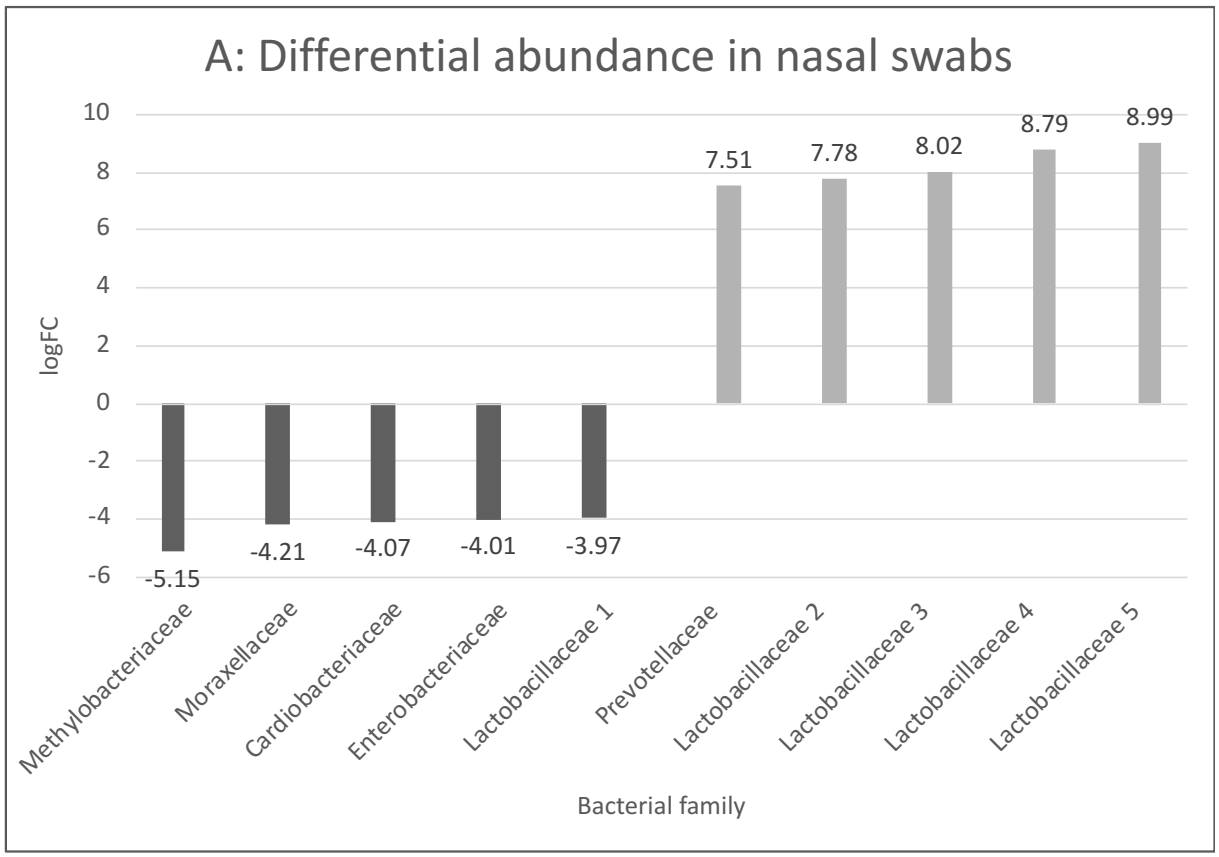


Figure 3: Representative samples for the nasal (A) and faecal (B) bacteriome
Representative samples (medoid samples) calculated for each pre-sampling farm for both sampling time points, pre and post consolidation, of the herds.
The different phyla are indicated by colour: Proteobacteria, Firmicutes, Bacteroidetes, others.

3.1.2 Differential abundance

To compare the change of the bacteriome before and after consolidation the paired differential abundance of the OTUs was calculated. The bacteria with the largest significant log fold changes upon consolidation are shown in *Figure 4*. Among these are several OTUs belonging to the same bacterial family. Deeper taxonomic classifications were not reliable with the 16S sequencing data of this study. In nasal swabs 82 OTUs were significantly less abundant and

347 more abundant after consolidation (P<0.05). In faecal samples 149 OTUs were significantly less abundant and 74 more abundant (P<0.05) after consolidation. The number of different bacterial families which underwent a large change upon consolidation is higher in nasal swabs than in faecal samples. The fold change is larger in nasal swabs than in faecal samples, meaning a larger shift occurred in the nasal bacteriome than in the faecal bacteriome.



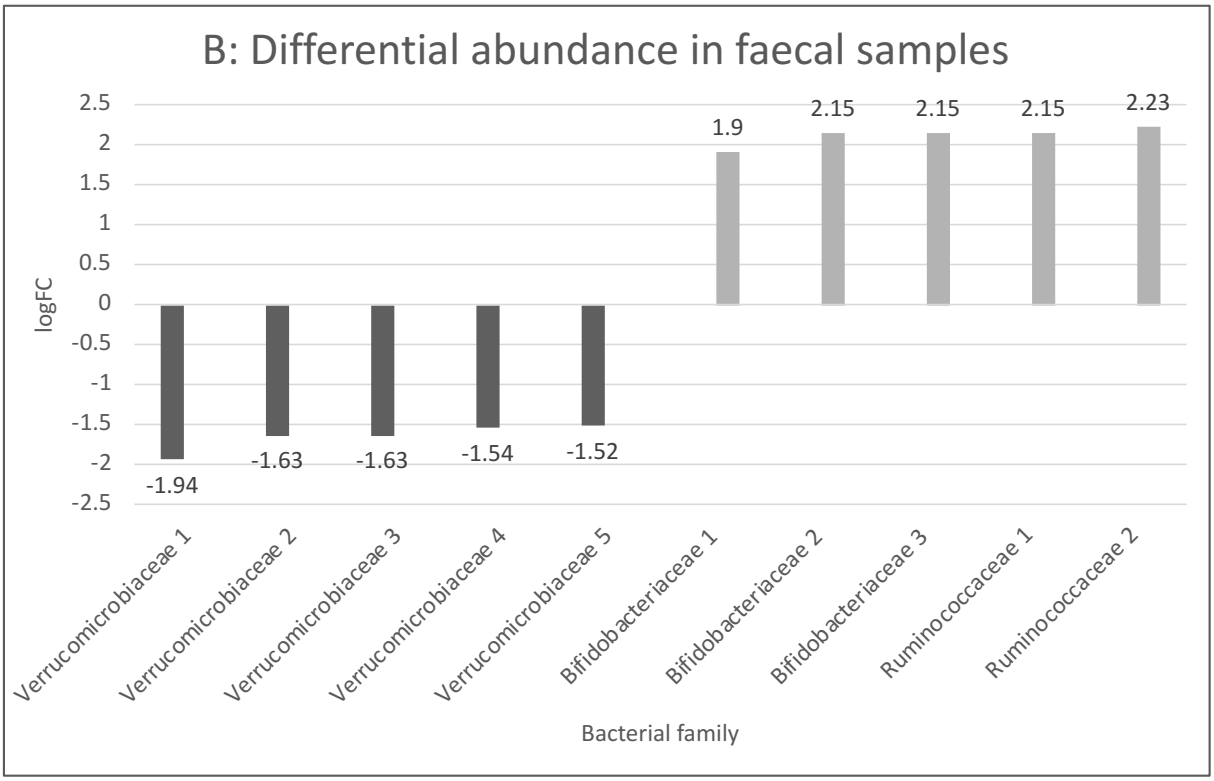


Figure 4: Differential abundance
Bacterial families with the largest log fold changes (logFCs) in nasal swabs (A) and faecal samples (B) are shown in this figure. A negative log FC means a decrease, a positive log FC an increase in abundance upon consolidation of the herds. Different OTUs of the same bacterial family are numerically labelled.

A dendrogram was created according to similarity of bacteriomes *(Figure 5 A)*. In nasal swabs (left side panels of *Figure 5*) a vertical pattern is visible, which means that changes of the abundance are associated to individual samples. Rearranging the order of the samples according to main herd *(Figure 5 B)* or pre-sampling herd (*Figure 5 C),* rather than to similarity of bacteriomes, shows that the changes (increase/decrease) are not herd-specific, only sample-related. In contrast, in faecal samples (right side panels of *Figure 5*) a horizontal pattern is visible indicating that the change of abundance is associated to individual OTUs. Likewise, as in nasal swabs, the changes are not associated to main or pre-sampling herds (*Figure 5 B* and *C*). In addition to main and pre-sampling farm, also birth farm, alpine summer farm, age, animal hospital stays or cattle market visits (special farms), born at the animal hospital or further farms the animals stayed on were used to rearrange the heat map (data not shown). None of these factors seem to be responsible for the clustering seen in the dendrogram. Additionally, no indication was found that laboratory data, like samples prepared together or similar DNA content after amplification or library preparation, might be responsible for the clustering. The clusters were not only visualized but also tested if they can be explained by a factor mentioned above. Besides Strickhof-Lindau being in only one of the clusters if only two clusters were

formed, none of the other variables tested showed a clear association with the clusters (i.e., being classified as "confirmed").

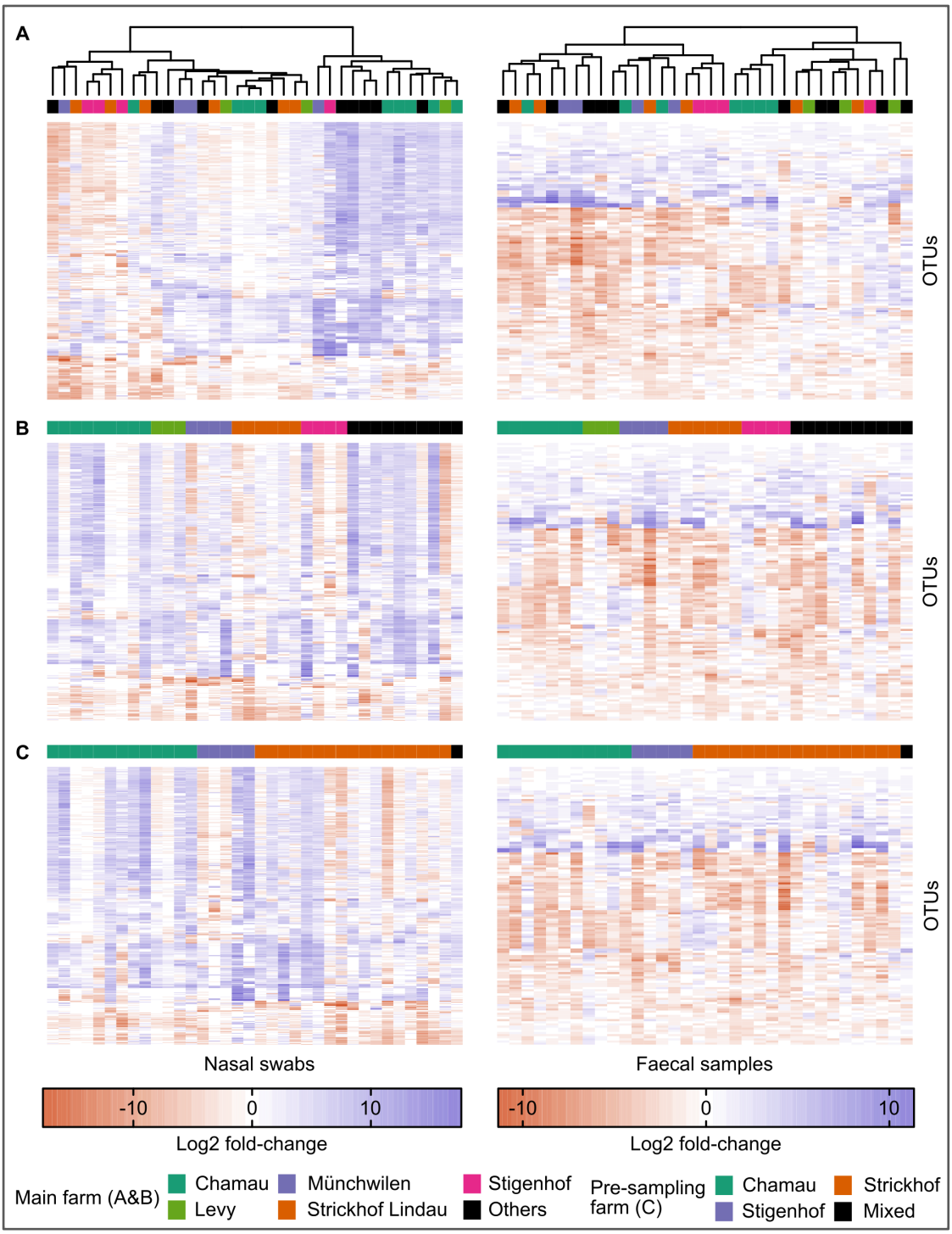


Figure 5: Heat maps for the paired differential abundance
OTUs with significant log fold changes in paired differential abundance, comparing samples after to before consolidation, are shown in the heat maps above. The x-axis shows the individual samples, the y-axis the OTUs found. Red colour shades indicating significantly less reads after consolidation, blue colour shades indicating significantly more reads after consolidation. A: Samples are arranged according to the dendrogram, which shows clusters of similar bacteriomes; B: Samples are arranged according to main farms; C: Samples are arranged according to pre-sampling farms.
The heat maps were generated by MWSchmid GmbH.

The calculated log fold changes from before to after consolidation of the significant OTUs were averaged for the bacterial families. Further, we tested if OTUs with an increased ("up") or decreased ("down") abundance upon consolidation were enriched or depleted for a certain taxonomic group *(Figure 6)*. Meaning, in the group of enriched OTUs ("up") and the group of depleted OTUs ("down") a certain amount of OTUs for each taxum is expected. If this number deviates from the expected one, the group is enriched or depleted for this taxum. Enriched/depleted means that in a certain taxum there were more/less OTUs found than it would be expected according to a random distribution.

In nasal swabs *Staphylococcaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillaceae* and *Moraxellaceae* became more abundant upon consolidation. *Staphylococcaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Lactobacillaceae* were enriched from the OTUs with increased abundance after consolidation. Hence, they were more often reacting positively to the consolidation than expected by chance. In contrast, *Moraxellaceae* were depleted from the OTUs with increased abundance after consolidation. Hence, they were less often reacting positively to the consolidation than expected by chance. *Enterobacteriacea*e and OTUs of unknown families were significantly less abundant after consolidation. *Enterobacteriacea* were enriched from the OTUs with decreased abundance after consolidation, meaning they were more often reacting negatively to the union of the herds than expected by chance. OTUs of unknown families were depleted from the OTUs with decreased abundance after consolidation, meaning they were less often reacting negatively to the union of the herds than expected by chance.

In faecal samples, *Methanobacteriaceae*, *Lachnospiraceae*, *Bifidobacteriaceae* and OTUs of unknown families were significantly more abundant after consolidation. From the OTUs with increased abundance *Methanobacteriaceae*, *Lachnospiraceae* and *Bifidobacteriaceae* were enriched, therefore more often reacting positively to the consolidation than expected by chance. OTUs of an unknown family were depleted and therefore reacting less often positively to the consolidation than expected by chance. *Verrucomicrobiaceae*, *Bacteroidaceae* and *Ruminococcaceae* were less abundant after the union of the herds. *Verrucomicrobiaceae* and *Bacteroidaceae* were enriched, thus more often reacting negatively to the consolidation than expected by chance, and *Ruminococcaceae* were depleted, thus reacting less often negatively to the consolidation than expected by chance.

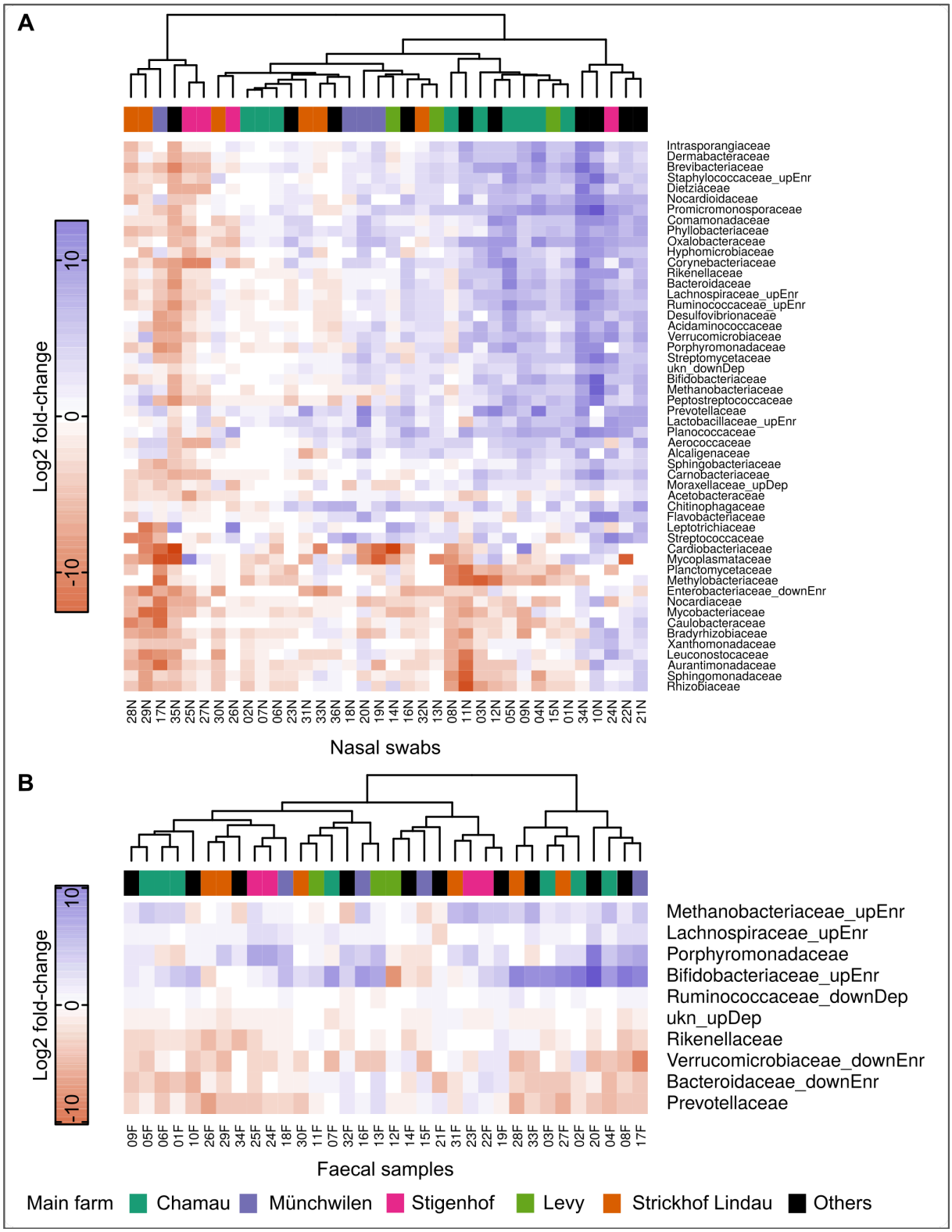


Figure 6: Paired differential abundance averaged across family
Only OTUs with a significant differential abundance are shown for nasal swabs (A) and faecal samples (B).
Up/down: higher/lower abundance of an OTU after consolidation
Enr/depl: enriched/depleted for a certain taxum; E.g., among all samples with an increased abundance (up) are a certain amount of each taxum expected if the distribution is random. If the number doesn't correlate with the expected number, the group "up" is enriched or depleted for this taxum.
The heat maps were generated by MWSchmid GmbH.

3.1.3 Sample specificity

To assess if there are certain OTUs which spread from a few animals to most animals upon consolidation or OTUs which vanished upon herd mixing, we calculated the sample specificity for each OTU. For six OTUs in nasal swabs there were not enough data available to calculate the sample specificity. There were no OTUs found which spread among the animals upon consolidation of the herds or which were abundant before and were only found in specific samples after consolidation *(Figure 7)*. In nasal swabs most OUTs were relatively sample specific before and also after consolidation. In faecal samples the majority of OTUs were quite abundant in most samples before and after consolidation.

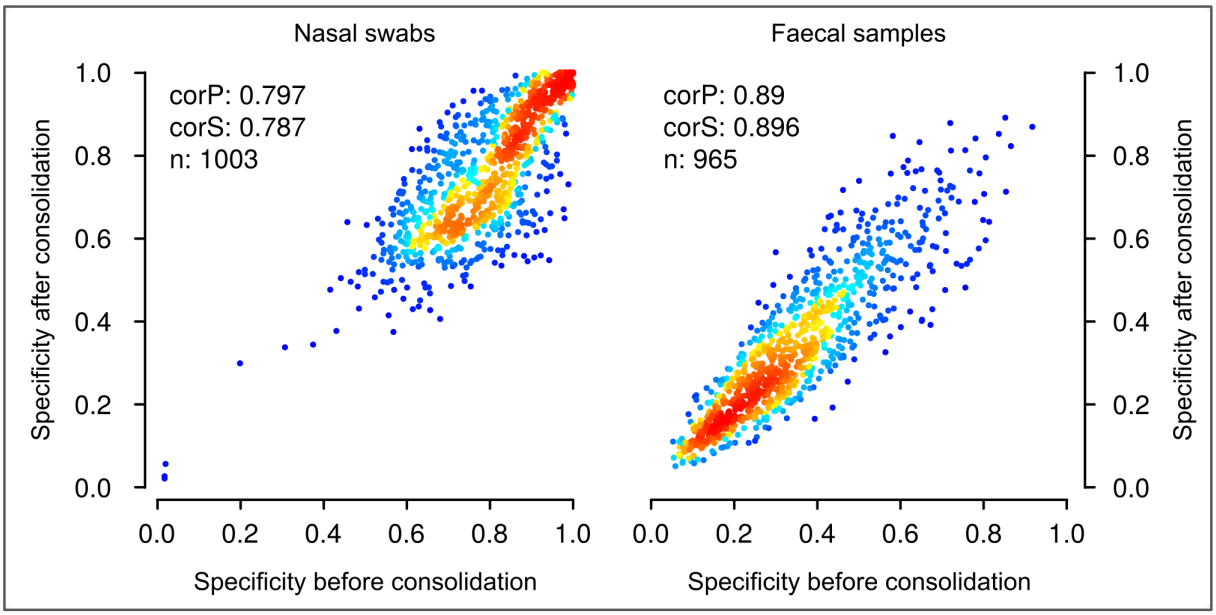


Figure 7: Sample specificity of the OTUs
Each dot represents an OTU. 0.0 means an OTU is equally abundant in all samples. 1.0 means an OTU is specific for one sample. Dots in the right-hand lower corner would stand for OTUs that changed from being specific to abundant upon consolidation and dots in left-hand upper corner for OTUs that were abundant and became specific with mixing of the herds.
This figure was generated by MWSchmid GmbH.

3.1.4 Diversity

To assess the diversity of the bacteriome the following indices were determined: species richness (SR), biodiversity (BD), effective richness (ER), Pielou's evenness (PE).
The four indices were calculated regarding two factors: group (sample pool) and the time of sampling (pre or post consolidation). *Table 6* shows an overview of the changes of the diversity indices upon consolidation.

The SR describes the number of species found, but not their abundance. There were no significant differences in SR in nasal swabs and in faecal samples. There was a tendency to a

decreasing SR upon mixing of the herds in nasal swabs and an increasing trend in faecal samples.

The BD index considers the number of OTUs and their abundance. In nasal swabs no significant differences in BD could be observed, neither between groups nor within a group between the first and the second sampling. But there was a tendency towards a decreased BD upon consolidation of the herds. In faecal samples there was a significant difference in BD between groups (P=0.0095) and a significant decrease (P=0.0112) of the BD within a group upon moving to the AgroVet-Strickhof farm.

The ER is the exponent of the BD. It describes the effective number of species present in a sample, in comparison to an index value which just describes the diversity but is not an actual species count. There were significant differences in nasal swabs (P=0.0086) and faecal samples (P=0.0082) in ER between different groups. However, within the groups there was no significant change in ER upon consolidation in nasal swabs, but a decreasing tendency. In faecal samples there was a significant decrease (P=0.012) of ER within the groups upon consolidation.

The PE measures the balance between the OTUs found, how equally they are represented in a sample. In nasal swabs there were no significant differences in PE between groups. Upon mixing of the herds, a trend towards decreased PE within groups was visible, but no significant changes. In faecal samples there were significant differences in PE (P=0.0024) between groups and a significant decrease upon consolidation (P=0.0053) within groups.

Diversity indices	Species richness	Biodiversity	Effective richness	Pielou's evenness
Nasal swabs	↓	↓	↓*[1]	↓
Faecal samples	↑	↓*	↓*	↓*

Table 6: Diversity indices
Overview of the direction of change upon consolidation of the four calculated diversity indices. Apart from effective richness in nasal swabs, the significance was the same for the indices between groups as well as within the groups upon consolidation.
↑/↓: increase/decrease in the respective diversity index upon consolidation
**: significant change in the diversity index upon consolidation*
[1]: only significant between different groups and not within the groups comparing before and after consolidation

To describe the influence of the group and the time of sampling on the diversity, the variation in diversity by these factors was calculated for the different indices *(Figure 8)*. We compared the difference in diversity among the different groups to the difference in diversity between the pre and post sample of the same group. For all indices, for the nasal and faecal bacteriome, the group had a much stronger influence on the diversity than the sampling time, meaning the

percentage of variation in diversity is higher between the different groups than among a group comparing the pre and post consolidation sample.

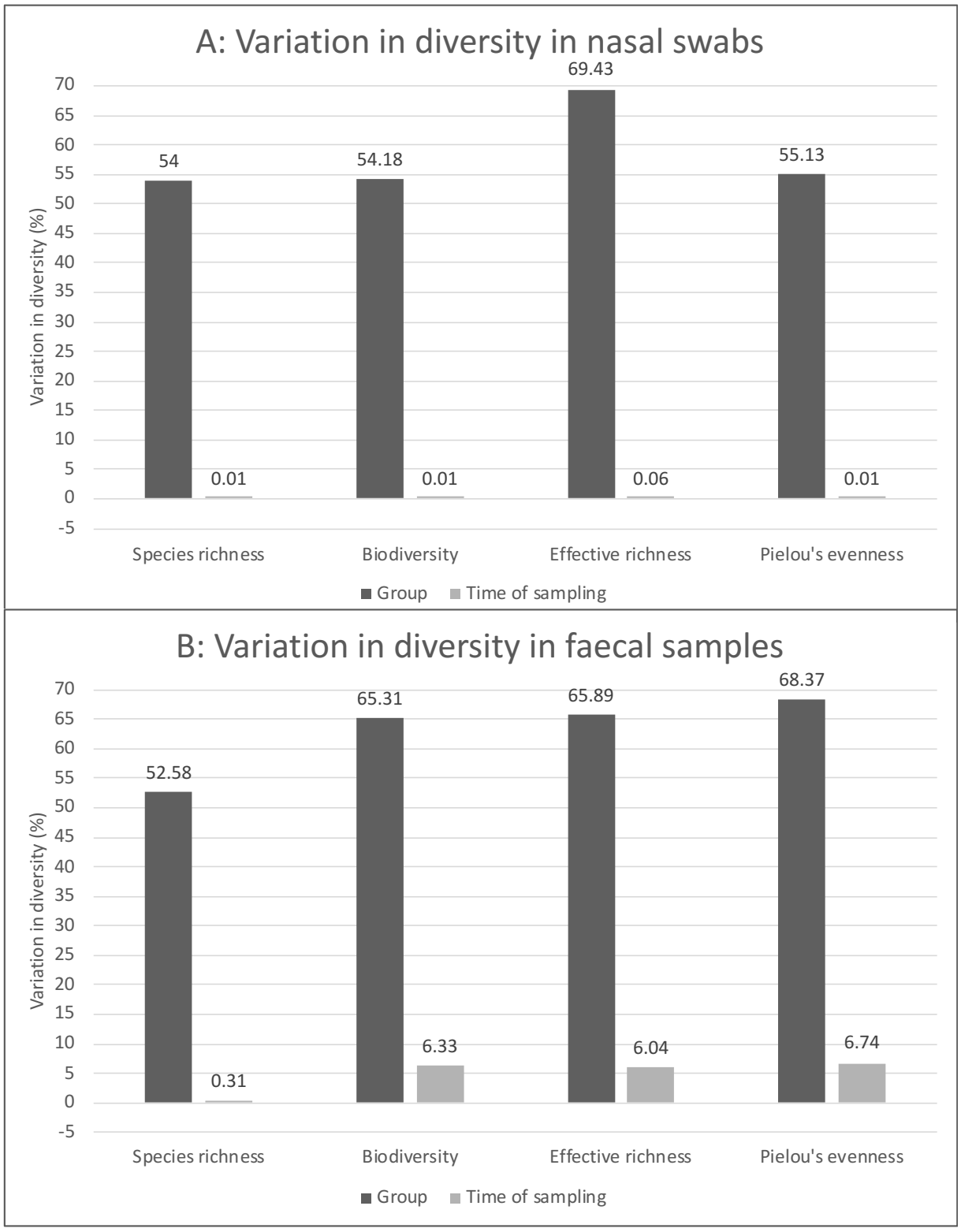


Figure 8: Variation in diversity
For all four diversity indices (species richness, biodiversity, effective richness, Pielou's evenness) the variation in diversity was calculated for nasal swabs (A) and faecal samples (B). The variation in diversity describes the strength of the influence of a given factor on the diversity, i.e., the percentage a given factor influences the variation in diversity. The factors considered here are the group (sample pool) and the time of sampling (pre vs. post consolidation).

3.1.5 Similarity of the bacterial community structure between groups

To describe the bacterial community structure the Manhattan distance, as a dissimilarity measurement, was calculated (Aggarwal et al., 2001). Subsequently, it was checked if the distances can be explained by the factors group or time of sampling. It describes if the bacteriome of the different samples became more or less similar to each other upon consolidation.

In nasal swabs the bacterial community structure (Manhattan distance) of the groups was not significantly different from each other, neither before nor after consolidation. But comparing the bacterial community structure (Manhattan distance) pre and post consolidation (time of sampling), it underwent a significant shift (P=0.001). In faecal samples the difference between groups (P=0.002) as well as the changes between the two sampling points (P=0.002) were significant. However, while this analysis showed significant differences of the sample similarity, the direction of this difference (more uniform or more divergent) for the different groups was not revealed. Therefore, we averaged the distances between the samples of the main farms before and after consolidation and visualized the change upon mixing of the herds *(Figure 9)*. The nasal bacteriome structure got more similar in half of the herds and less similar in the other half upon mixing of the herds. In the faecal samples four out of six herds showed a more similar bacterial structure after consolidation than before. It seems that the consolidation had a more unifying influence on the faecal bacteriome of the herds than on the nasal bacteriome.

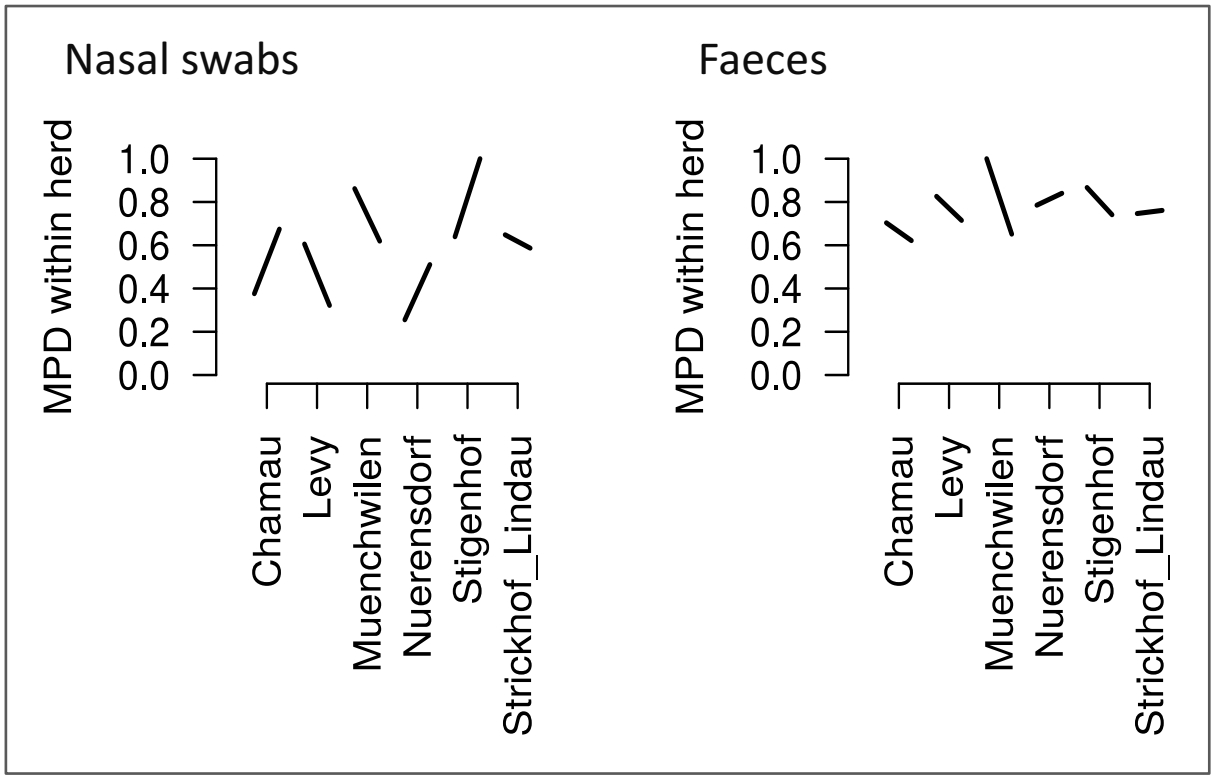


Figure 9: Change in bacterial composition per main farm
The figure shows the change in the similarity of the bacterial composition upon consolidation (Manhattan distance). The results are shown in the mean pairwise distance (MPD) (Webb et al., 2002) for the main farms. An increasing MPD means the bacteriome composition of the different samples of the same main farm got more divergent, a decreasing MPD means the bacteriome of the samples of the same main farm got more uniform upon consolidation.
This figure was generated by MWSchmid GmbH.

Additionally, the strength of the influence on the community structure for the factors group and time of sampling was computed for the Manhattan distance, meaning to what extend a factor is responsible for the change in bacteriome composition upon consolidation. In both, nasal swabs and faecal samples, the sample had the stronger impact on the community structure than the time of sampling *(Figure 10)*.

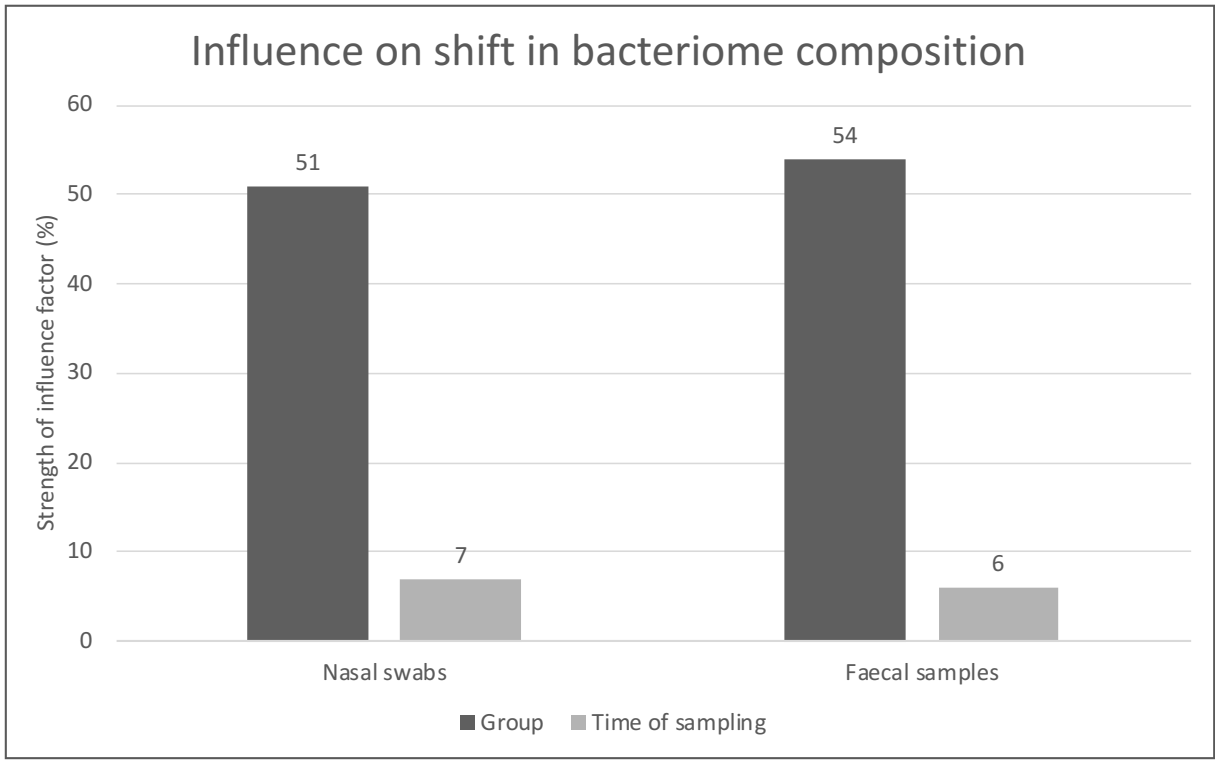


Figure 10: Influence on shift in bacteriome composition
This figure shows the strength (y-axis) of the two influence factors considered, group and time of sampling. For both sample types the group had a larger influence on the change in bacteriome composition than the time of sampling.

3.2 Virome

In total, 78 nasal swab samples and 74 faecal samples were sequenced for their virome. In the nasal swab samples a mean of 2,211,925.5 raw reads were generated, in faecal samples a mean of 3,151,278.5 reads.

3.2.1 Spectrum of viruses found before and after consolidation

In nasal swabs eight and in faecal samples six different viral families were detected. *Table 7* shows an overview of the viral families and genera found, the number of reads and the corresponding best coverage. Phages and known plant viruses were not included in the analysis. The families *Astroviridae*, *Circoviridae*, *Genomoviridae*, *Parvoviridae, Picornaviridae* and *Smacoviridae* were found in nasal swabs and faecal samples. Reads of the families *Papillomaviridae* and *Pneumoviridae* were only found in nasal swabs. Mainly in nasal swabs, reads of several viruses belonging to the group of circular Rep-encoding single-stranded DNA viruses (CRESS DNA viruses), not assigned to a family, were detected. Among the detected viral families, also *Circoviridae*, *Genomoviridae* and *Smacoviridae* belong to the group of CRESS DNA viruses.

Number of viral reads		Nasal swabs		Faecal samples		Best genome coverage (%)
Family	**Genus (where applicable)**	**Pre**	**Post**	**Pre**	**Post**	
Astroviridae	Mamastrovirus	14	0	0	12	5.00
Circoviridae[1]	unclassified Circoviridae	10	8	2736	604	69.37
Genomoviridae[1]	*Gemycircularvirus*	1510	74	518	12	87.84
	Gemygorvirus	14	24	0	0	63.43
	Gemykibivirus	16	0	0	0	16.90
	Gemykrogvirus	0	0	12	0	24.28
	unclassified Microviridae	0	0	1084	1934	65.02
not assigned (n/a) CRESS DNA viruses[1,2]	*unclassified ssDNA viruses*	102	224	0	26	82.09
	unclassified viruses	0	8	0	0	38.11
	uncultured marin virus	12	4	0	0	28.43
	uncultured virus	1182	1664	0	0	88.84
Papillomaviridae	Dyoxipapillomavirus	0	4	0	0	5.81
	Epsilonpapillomavirus	0	34	0	0	6.09
	unclassified Papillomaviridae	6	0	0	0	5.25
Parvoviridae	*Bocaparvovirus*	42	501872	0	224	100.00
	Dependoparvovirus	0	4	0	0	68.36
Picornaviridae	*Aphthovirus*[3]	0	13	0	0	11.28
	Enterovirus	0	0	34	20	15.45
	Hunnivirus	0	0	24	0	29.50
	Kobuvirus	0	0	472	372	42.86
Pneumoviridae	*Orthopneumovirus*	2	0	0	0	61.84
Smacoviridae[1]	*Bovisamcovirus*	0	6	1162	1730	42.35
	Porprismacovirus	0	0	162	190	44.38

Table 7: Number of viral reads
Overview over the viral reads detected per family and genus. In the last column the best genome coverage, in percentage, is given for the respective viral genus. The viral genera in italic are further discussed.
[1] circular Rep-encoding ss DNA viruses
[2] circular Rep-encoding ss DNA viruses, not assigned to an official family
[3] not included in further analysis

3.2.1.1 Number of reads

An overview of all viral reads detected in the samples and included in the analysis is given in *Table 7*. Only the genus *Aphthovirus* was not included in the further analysis as these reads were only detected with a custom-made database as explained in Material and Methods (chapter *2.6.2*). This made it impossible to compare the read counts and coverages found for *Aphthovirus* to the results of the other genera that were detected using the VirDetect App on the Sushi platform.

In nasal swabs a total of 506,849 viral reads were detected of which 492,488 were assigned to one virus in a single pool as described in the next paragraph. Even when these reads are not counted, still more viral reads were detected in nasal swabs, 14,361 reads, than in faecal samples, 11,328 reads. *Astroviridae* and *Genomoviridae* were equally present in nasal swabs and faecal samples. *Parvoviridae* were clearly more prevalent in nasal swabs, but 99.93% of the reads were found in two samples (sample Nr. 14 and sample Nr. 20) from after consolidation. In sample Nr. 14 three nasal swabs from the main farm "Levy" respectively pre-sampling farm "Strickhof" were pooled. Sample Nr. 20 is a pool of three nasal swabs from the main farm "Münchwilen" and also the pre-sampling farm "Strickhof". Taking these two samples not into account, also *Parvoviridae* were equally distributed among nasal swabs and faecal samples. Not assigned CRESS DNA viruses, *Papillomaviridae* and *Pneumoviridae* were more frequent in nasal swabs, *Circoviridae*, *Picornaviridae* and *Smacoviridae* more common in faecal samples.

Most reads by far were assigned to the genus *Bocaparvovirus*, species *Ungulate bocaparvovirus 6*. It was detected in twelve nasal swab samples with a total of 501,914 reads. The majority of reads, namely 492,488, were found in a single sample, Nr. 20 after consolidation. In this sample the full genome of an ungulate bocaparvovirus most closely related to ungulate bocaparvovirus 6 strain Mex-S22 was detected with a length of 5,060 bp. This was the only fully sequenced genome in this study.

Among the detected viral families, *Circoviridae*, *Genomoviridae*, not assigned CRESS DNA viruses and *Smacoviridae* belong to the group of CRESS DNA viruses *(Table 8)*. Therefore, this group makes up around half of the viral families found in the samples. Not assigned CRESS DNA viruses were mainly found in nasal swabs, *Circoviridae*, *Genomoviridae* and *Smacoviridae* were more frequently detected in faecal samples.

Nasal swabs	**Family**	**Virus**	**Pre**	**Post**
	Circoviridae	Circoviridae sp.	10 (5%)	4 (3%)
		Dromedary stool-associated circular ssDNA virus	0 (0%)	4 (5%)
	Genomoviridae	Faecal-associated gemycircularvirus 1b	2 (3%)	0 (0%)
		Faecal-associated gemycircularvirus 2	8 (10%)	8 (8%)
		Faecal-associated gemycircularvirus 4	0 (0%)	2 (3%)
		Faecal-associated gemycircularvirus 6	1440 (33%)	28 (18%)
		Meles meles fecal virus	14 (13%)	24 (18%)
		Mongoose feces-associated gemycircularvirus b	16 (3%)	0 (0%)
		Porcine feces-associated gemycircularvirus	16 (8%)	32 (5%)
		Pteropus associated gemycircularvirus 3	42 (13%)	4 (5%)
		Sewage-associated gemycircularvirus 1	2 (3%)	0 (0%)
	not assigned CRESS DNA viruses	*CRESS virus sp.*	102 (46%)	218 (49%)
		Rodent stool-associated circular genome virus	0 (0%)	2 (3%)
		Sewage-associated circular DNA virus-14	0 (0%)	8 (5%)
		Sewage-associated circular DNA virus-17	0 (0%)	4 (5%)
		Uncultured marine virus	12 (8%)	4 (5%)
		Uncultured virus	1182 (90%)	1664 (92%)
	Smacoviridae	*Bovine associated bovismacovirus 1*	0 (0%)	6 (5%)
Faeces	**Family**	**Virus**		
	Circoviridae	Cattle blood-associated circovirus-like virus	212 (5%)	30 (3%)
		Circoviridae sp.	24 (3%)	0 (0%)
		Dromedary stool-associated circular ssDNA virus	2500 (35%)	574 (22%)
	Genomoviridae	*Alces alces faeces associated microvirus MP11 5517*	1084 (68%)	1934 (59%)
		Caribou feces-associated gemycircularvirus	12 (3%)	0 (0%)
		Chicken stool-associated gemycircularvirus	82 (11%)	0 (0%)
		Faecal-associated gemycircularvirus 6	134 (16%)	0 (0%)
		Porcine feces-associated gemycircularvirus	184 (24%)	12 (3%)
		Pteropus associated gemycircularvirus 3	118 (14%)	0 (0%)
	not assigned CRESS DNA viruses	*CRESS virus sp.*	0 (0%)	26 (5%)
	Smacoviridae	Camel associated porprismacovirus 1	0 (0%)	38 (3%)
		Camel associated porprismacovirus 2	30 (5%)	152 (14%)
		Bovine associated bovismacovirus 1	1162 (68%)	1730 (84%)
		Porcine associated porprismacovirus 10	132 (3%)	0 (0%)

Table 8: Circular Rep-encoding single-stranded DNA viruses (CRESS DNA viruses)
This table provides an overview of the different CRESS DNA viruses detected in this study (i.e., the best matching references from the database), the overall read counts and the prevalence (in brackets) of positive samples. The viruses in italic are further discussed.

The results were also specifically checked for known pathogenic bovine viruses. There were two potentially pathogenic viruses found in the nasal swab samples and none in faecal samples. In the nasal swab sample Nr. 39 pre consolidation two reads of bovine respiratory syncytial virus (BRSV) were found. This sample contained only material of one cow "Jara", a cow from the main farm "übrige Betriebe" and the pre-sampling farm "Strickhof". In the sample Nr. 16, also main farm "übrige Betriebe" and pre-sampling farm "Strickhof", was also specimen of "Jara" sequenced, but no reads of BRSV could be detected. "Jara" is one of two cows who was sequenced individually as well as in a pool to get an impression of how much sensitivity is lost upon pooling of the samples.

By screening the samples against a database containing all complete viral genomes available on GenBank, 13 reads of *Bovine rhinitis B virus* were found in nasal sample Nr. 16 after consolidation. The viral sequence was also present in the database used on the sushi platform, but no reads of *Bovine rhinitis B virus* were detected by the platform.

Among the cows sampled was a set of twins. They were first sampled on the pre-sampling farm "Strickhof" and belong to the main farm "Levy". Their sample material was sequenced in a pool with a total of four animals but also individually. The results are shown *Figure 11*. In the individual nasal swabs of the twin pair and the pool pre consolidation, not assigned CRESS DNA viruses and *Genomoviridae* were found. Post consolidation only not assigned CRESS DNA viruses were detected in the individual twin samples. In the pool post consolidation most reads were assigned to P*arvoviridae* and a few to not assigned CRESS DNA viruses. In the pre and post consolidation faecal samples *Smacoviridae* and *Genomoviridae* were detected in the pool. In the individual twin samples only *Smacoviridae* reads were detected. The individual twin set samples of both sample types were very similar to each other. However, in the respective pool the diversity seems to be slightly higher. Also, in two of the individual twin samples no viral reads could be detected, which was rarely the case in a pooled sample.

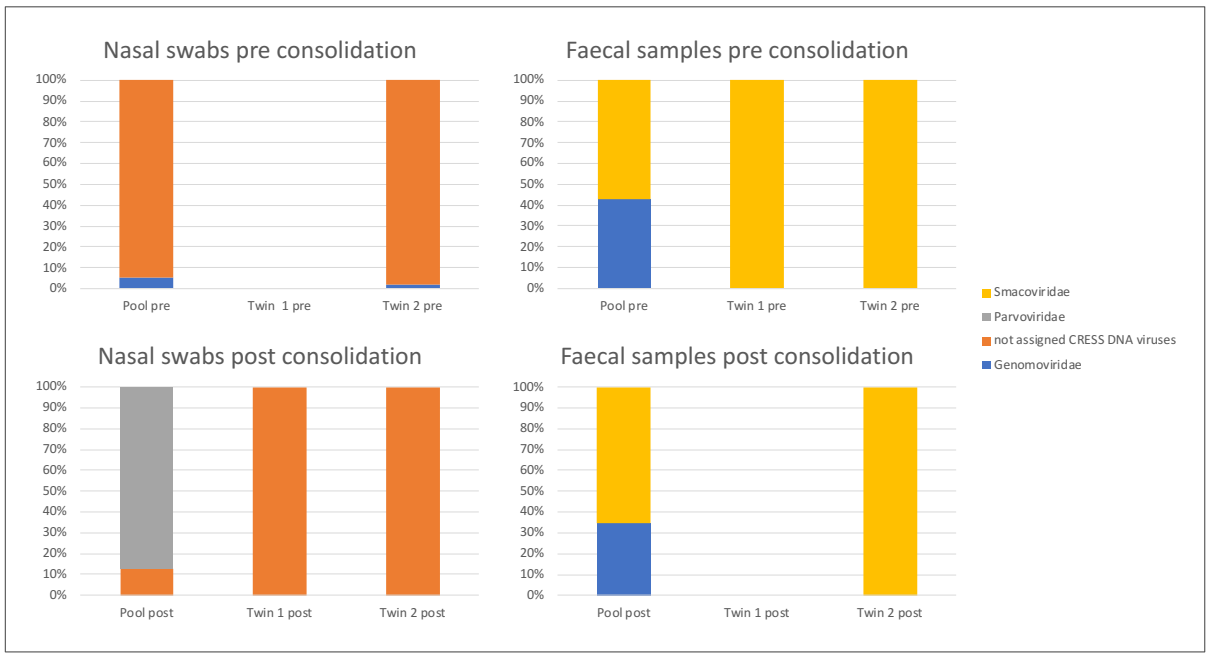


Figure 11: Set of twins
The figure shows the distribution in percentage of viral families found in the twin samples and their respective pools.

Due to the large number of animals and samples included in the study, samples had to be pooled prior to sequencing for economic reasons. To assess a potential loss of sensitivity in virus detection by pooling, four samples, one nasal swab and one faecal sample from before and after consolidation, were sequenced pooled as well as individually. The results are shown in *Figure 12*. In the nasal samples analysed, not assigned CRESS DNA viruses, *Genomoviridae* and *Pneumoviridae* were found. Not assigned CRESS DNA viruses and *Genomoviridae* reads were found pre and post consolidation in a similar distribution. In the pool post consolidation no viral reads could be detected. The two reads of *Pneumoviridae* present before consolidation in the individual sample were lost upon pooling. In faecal samples in both pools, pre and post consolidation, the percentage of *Smacoviridae* and *Genomoviridae* reads was increased compared to the respective individual sample. The percentage of *Circoviridae* reads on the other hand was reduced in the pooled samples.

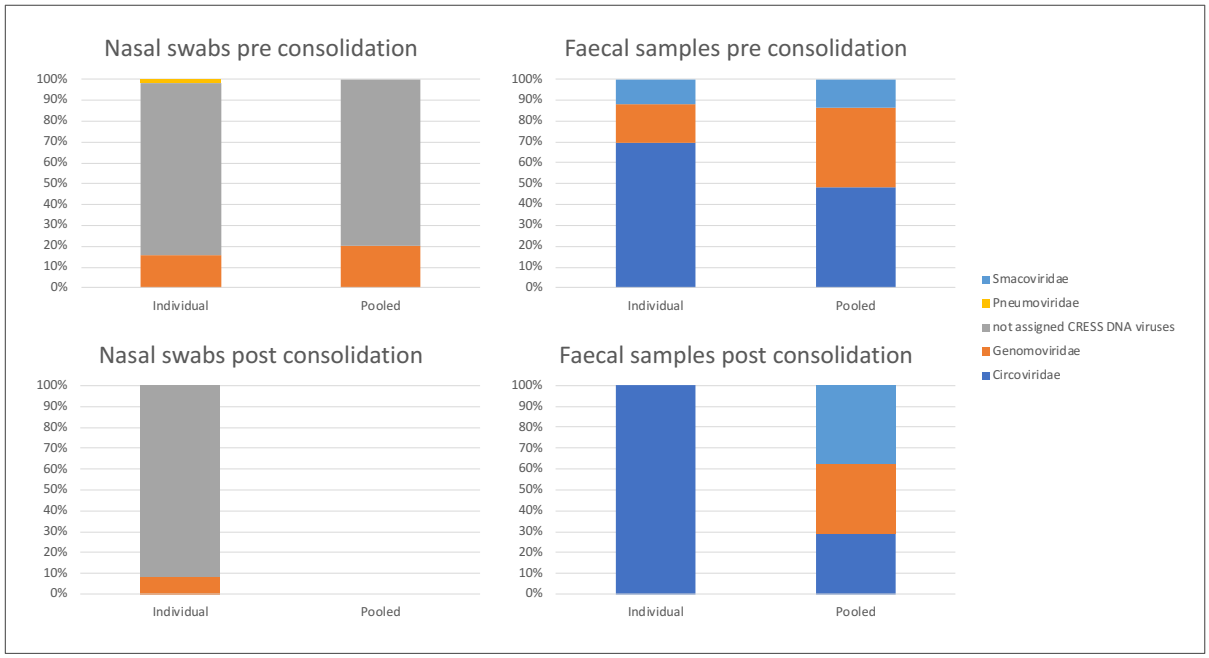


Figure 12: Individual samples
The figure shows the sequencing results of a nasal swab and a faecal sample, pre and post consolidation of an individual animal and its respective pool.

3.2.1.2 Prevalence of viruses

Papillomaviridae and *Pneumoviridae* were only found in nasal swabs, the other viral families mentioned in *Table 9* were at least detected in one nasal swab and one faecal sample. *Astroviridae* were equally present among nasal swabs and faecal samples. *Genomoviridae* were well represented in nasal swabs and faecal samples, but more frequent in faecal samples. Also, the spectrum of genera detected was different in the two samples types.

Not assigned CRESS DNA viruses and *Parvoviridae* were more frequent in nasal swabs, and *Circoviridae, Picornaviridae* and *Smacoviridae* were more often detected in faecal samples.

Prevalence of viral reads		Nasal swabs		Faecal samples	
Family	**Genus (where applicable)**	**Pre**	**Post**	**Pre**	**Post**
Astroviridae	Mamastrovirus	3%	0%	0%	3%
Circoviridae	unclassified Circoviridae	5%	8%	43%	24%
Genomoviridae	Gemycircularvirus	51%	31%	43%	3%
	Gemygorvirus	13%	18%	0%	0%
	Gemykibivirus	3%	0%	0%	0%
	Gemykrogvirus	0%	0%	3%	0%
	unclassified Microviridae	0%	0%	68%	59%
not assigned (n/a) CRESS DNA viruses	unclassified ssDNA viruses	46%	51%	0%	5%
	unclassified viruses	0%	5%	0%	0%
	uncultured marin virus	8%	5%	0%	0%
	uncultured virus	90%	92%	0%	0%
Papillomaviridae	Dyoxipapillomavirus	0%	3%	0%	0%
	Epsilonpapillomavirus	0%	5%	0%	0%
	unclassified Papillomaviridae	3%	0%	0%	0%
Parvoviridae	Bocaparvovirus	18%	13%	0%	5%
	Dependoparvovirus	0%	5%	0%	0%
Picornaviridae	Aphthovirus	0%	3%	0%	0%
	Enterovirus	0%	0%	3%	3%
	Hunnivirus	0%	0%	3%	0%
	Kobuvirus	0%	0%	24%	22%
Pneumoviridae	Orthopneumovirus	3%	0%	0%	0%
Smacoviridae	Bovisamcovirus	0%	5%	68%	84%
	Porprismacovirus	0%	0%	8%	16%

Table 9: Prevalence of viral reads
The table shows the percentage of samples in which a viral genus was found. All 39 sequenced nasal swabs and 37 sequenced faecal samples were considered.

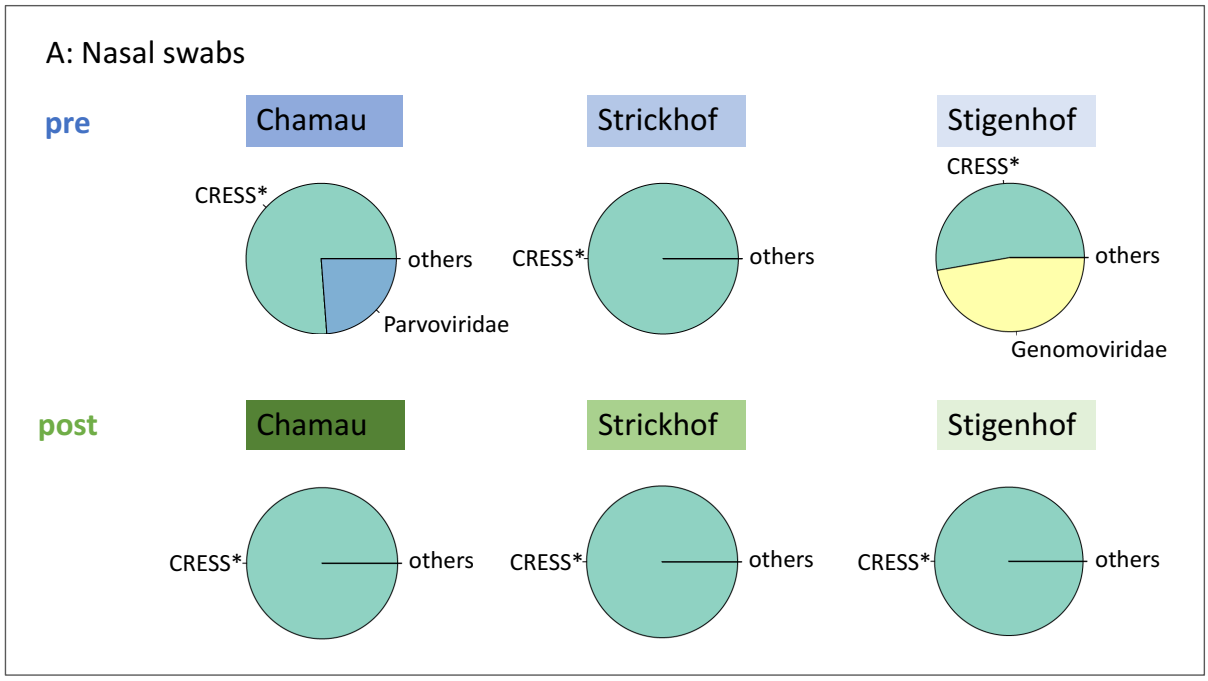
3.2.2 Differences between herds

3.2.2.1 Representative samples

To gain an overview of the typical virome composition, representative samples, so-called medoids (least dissimilarity between samples), were calculated for each original farm and each sampling timepoint *(Figure 13)*. In nasal swabs the reads predominantly found in the medoid sample belong to the group of not assigned CRESS DNA viruses. In the medoid pre consolidation sample of "Chamau" there were nearly a quarter *Parvoviridae* detected, and in the pre consolidation medoid sample of "Stigenhof" approximately half of the reads belong to the family *Genomoviridae*. In all three post consolidation medoid samples the majority of reads belong to the group of not assigned CRESS DNA viruses.

In the faecal medoid sample *Circoviridae*, *Genomoviridae* and *Smacoviridae* were predominant. In the "Chamau" medoid sample *Circoviridae* were mostly lost upon consolidation. In the representative "Strickhof" sample *Genomoviridae* and *Smacoviridae* were most frequent pre and post consolidation. In the medoid "Stigenhof" sample *Genomoviridae* were mostly lost upon consolidation.

Comparing the medoid samples of nasal swabs and faecal samples, the viral spectrum is quite different. Where in nasal swabs not assigned CRESS DNA viruses and some *Parvoviridae* and *Genomoviridae* reads are mostly present, in faecal samples *Circoviridae*, *Genomoviridae* and *Smacoviridae* are equally represented. In both sample types the representative samples show less diversity after consolidation.



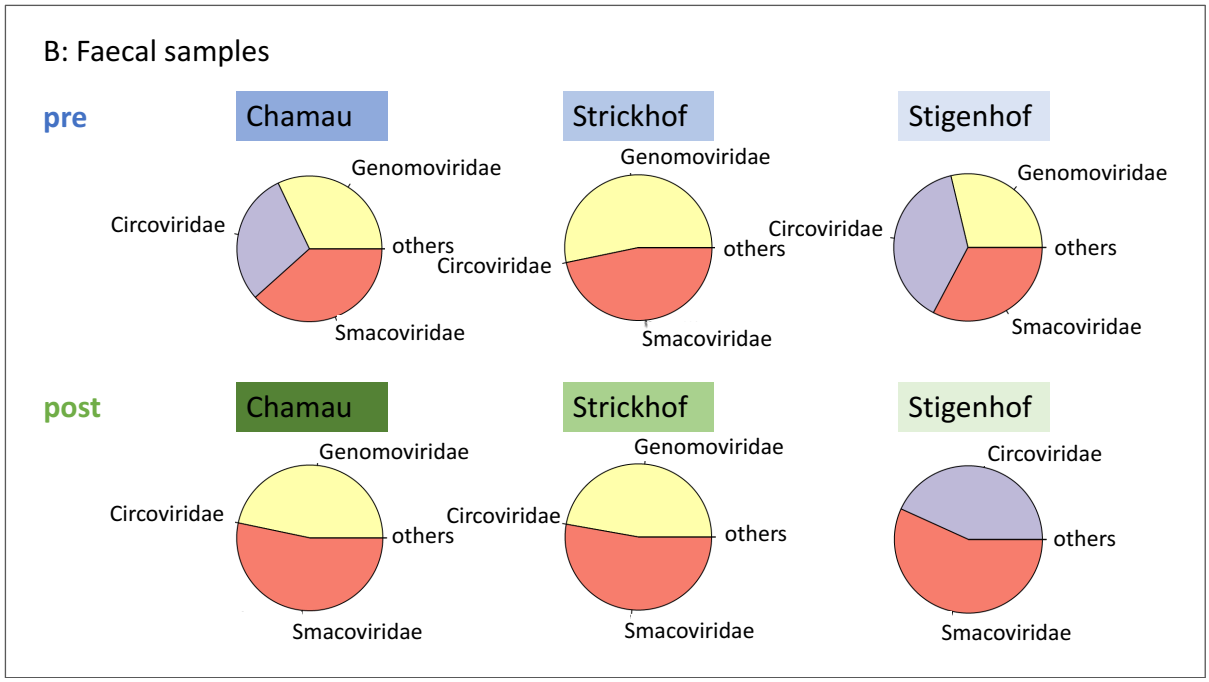


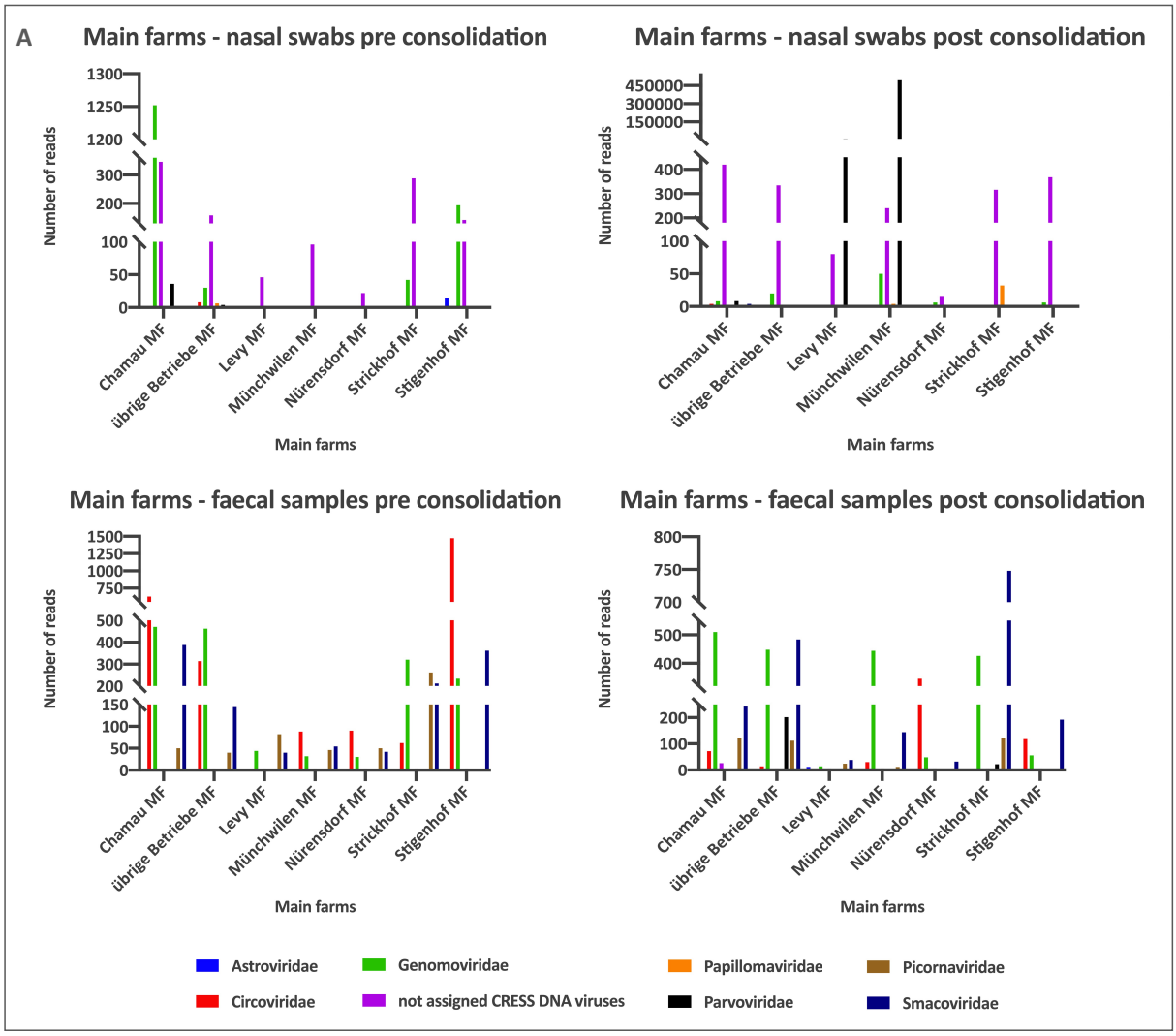
Figure 13: Representative samples (medoid) of the nasal (A) and faecal (B) virome
The medoid samples represent the typical virome composition for each pre-sampling farm pre and post consolidation.
**CRESS: not assigned CRESS DNA viruses*

3.2.2.2 Number of reads

For the following calculations 36 nasal pools and 34 faecal pools were included. The six samples which were additionally sequenced (two individual samples and four samples of twins) were not included, as these cows were already included in their respective pool. For the statistical analysis the Wilcoxon signed rank test was performed comparing the pre and post consolidation median read counts. *P*-values <0.05 were considered statistically significant.

An overview of the changes in viral read count upon consolidation is given in *Figure 14*. It shows the read counts pre and post consolidation for all main farms (MF) and pre-sampling farms (PF). In nasal swabs of all farms pre and post consolidation was a considerable amount of not assigned CRESS DNA viruses reads detected. "Chamau MF" and "PF" as well as "Stigenhof MF" and "PF" lost a lot of *Genomoviridae* reads upon consolidation. In "Levy MF", "Münchwilen MF" and "Strickhof PF" the number of *Parvoviridae* strongly increased upon consolidation.

In faecal samples much more changes in viral read count upon consolidation were detected. A fair amount of *Genomoviridae* reads could be detected in nearly all farms pre and post consolidation. In most farms there is a decrease in *Circoviridae* and an increase of *Smacoviridae* reads apparent upon consolidation. More details are shown in *Figure 14*.


A
Main farms - nasal swabs pre consolidation
Main farms - nasal swabs post consolidation
Main farms - faecal samples pre consolidation
Main farms - faecal samples post consolidation
Number of reads
Main farms
Chamau MF
übrige Betriebe MF
Levy MF
Münchwilen MF
Nürensdorf MF
Strickhof MF
Stigenhof MF
Astroviridae
Circoviridae
Genomoviridae
not assigned CRESS DNA viruses
Papillomaviridae
Parvoviridae
Picornaviridae
Smacoviridae

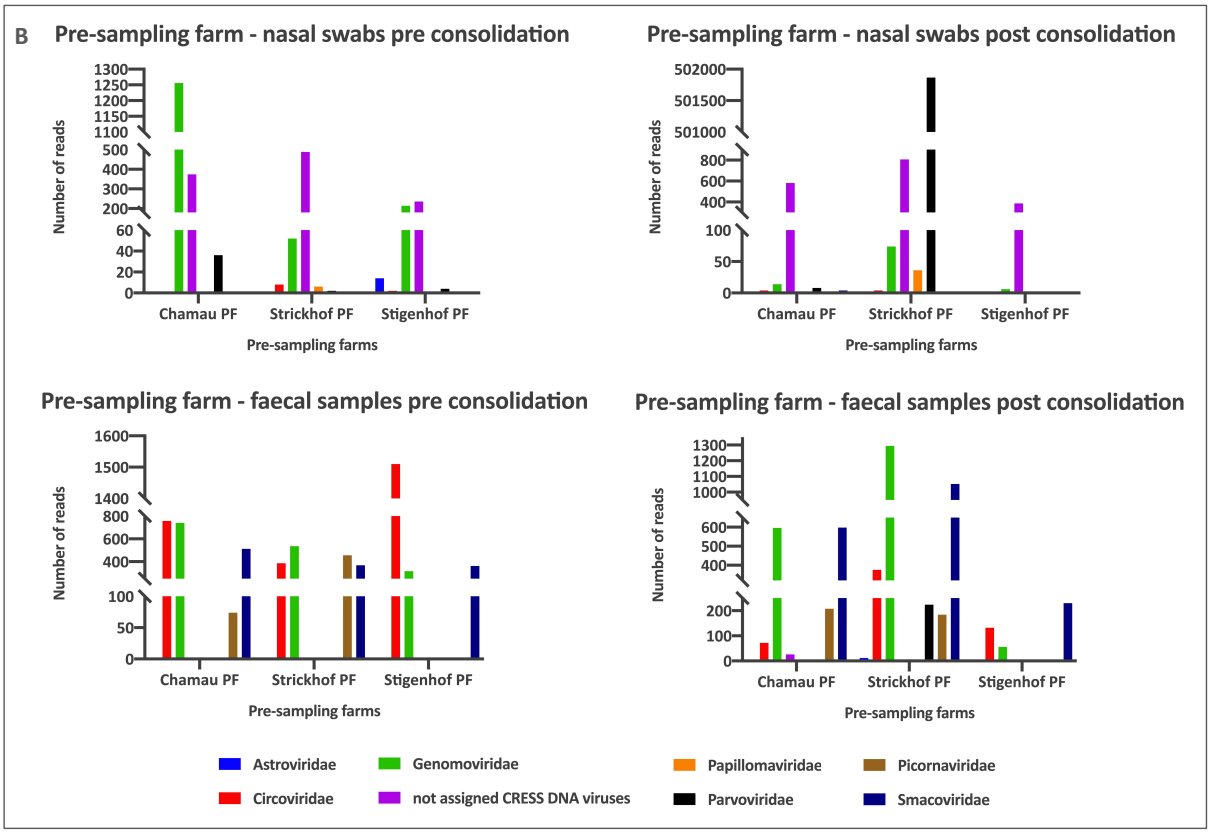


Figure 14: Number of viral reads
The figure shows the number of viral reads for each viral family and farm pre and post consolidation. In A the main farms and in B the pre-sampling farms are shown.
MF: main farm
PF: pre-sampling farm

In *Figure 15* only farms with significant changes in the viral read counts upon consolidation are shown. There was a significant decrease of *Genomoviridae* reads in nasal swabs of "Strickhof MF" upon consolidation (*P*=0.041). In faecal samples there was a significant decrease (*P*=0.042) in *Circoviridae* reads and a significant increase of *Smacoviridae* reads (*P*=0.027) in "übrige Betriebe MF". Further significant changes in faecal samples were a decrease in *Circoviridae* reads (*P*=0.046) in "Chamau PF" and a significant decrease (*P*=0.021) in *Picornaviridae* reads in "Strickhof PF". These numbers are based on absolute read counts. If calculating the same using relative read counts (in proportion to the total amount of reads per sample), the same viral families showed significant change in the same direction with slightly different *P*-values. In all other farms not mentioned in this paragraph, no significant changes in the virome could be observed upon consolidation.

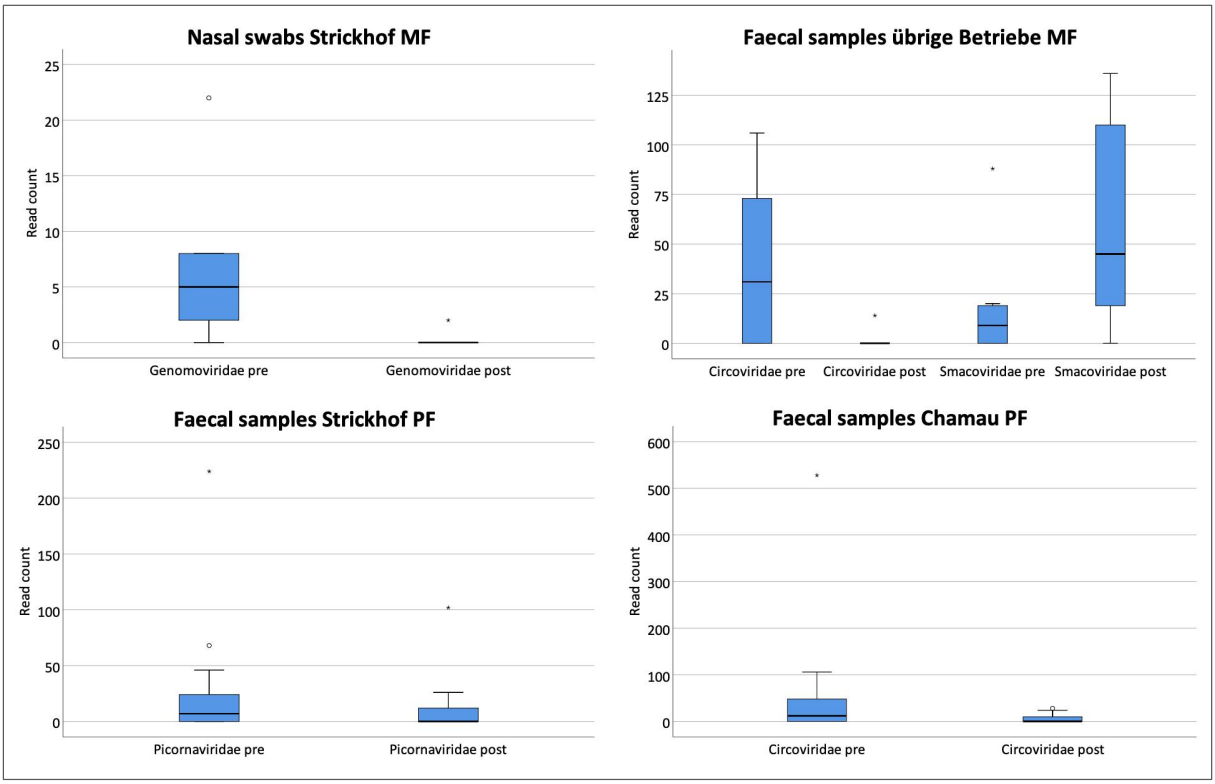


Figure 15: Significant changes in viral read count
This figure shows the farms with significant changes in read count of one or several viral families upon consolidation. For the statistical analysis the Wilcoxon rank sum test was performed using the mean read count shown on the y-axis.

3.2.2.3 Viral family prevalence

For each viral family the percentage of samples it was found in was calculated *(Figure 16)*. In nasal swabs the most common viruses were not assigned CRESS DNA viruses, *Genomoviridae* and *Parvoviridae* before and after consolidation. In faecal samples *Smacoviridae*, *Genomoviridae*, *Circoviridae* and *Picornaviridae* were the dominant viral families. Taking all sample pools into account, the changes in the nasal virome were less evident than the ones in the faecal virome, where a significant decrease in prevalence of *Circoviridae* (P=0.039) and a significant increase of *Smacoviridae* (P=0.039) upon mixing of the herds was detected. Calculating the prevalence on the individual farm level, a significant increase in proportion of positive pools was only found for *Smacoviridae* in faecal samples from the pre-sampling farm "Strickhof" (P=0.031). For the statistics proportions were compared with the McNemar test.

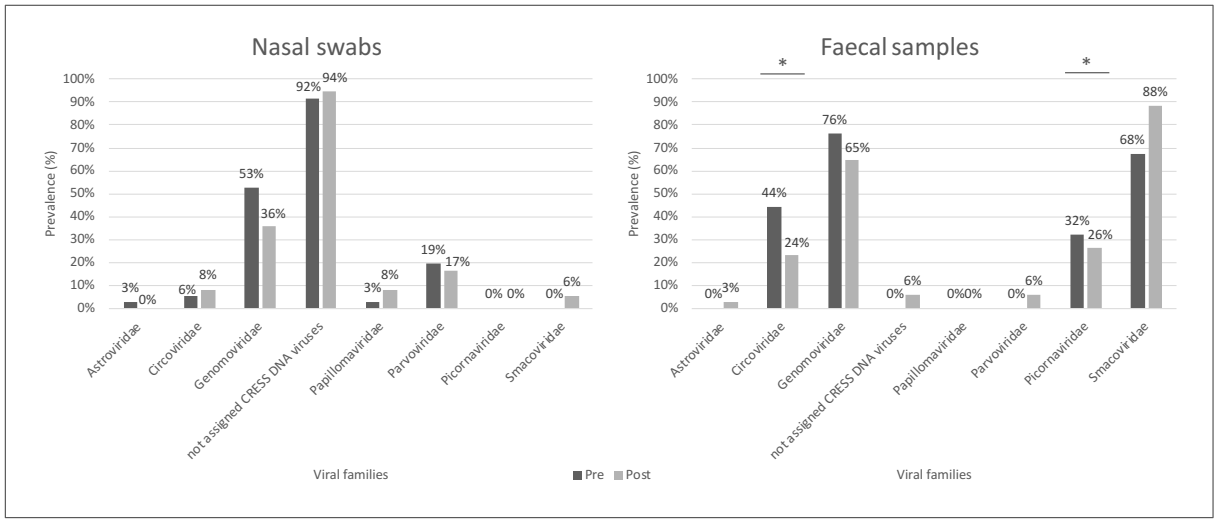


Figure 16: Prevalence of viral families
The figure shows the percentage of the sample pools in which a certain viral family was present. Proportions were compared with McNemar test, and significant changes of prevalence upon consolidation of the herds were marked with an asterisk (*).

3.2.3 Sample specificity

To assess if viruses spread or were lost upon consolidation, we analysed how specific viral families were for the sample pools before and after consolidation *(Figure 17)*. Compared to the bacteriome data, only relatively few virus families were detected and only in a limited number of samples, therefore only five viral families from nasal swabs and four from faecal samples could be considered for this analysis. In nasal swabs most viral families were quite sample specific and stayed sample specific upon mixing of the herds. Only not assigned CRESS DNA viruses were present in many samples before and after consolidation. In faecal samples *Genomoviridae* and *Smacoviridae* were widely spread among the animals, and *Circoviridae* and *Picornaviridae* were rather sample specific. The specificity did not change upon consolidation of the herds.

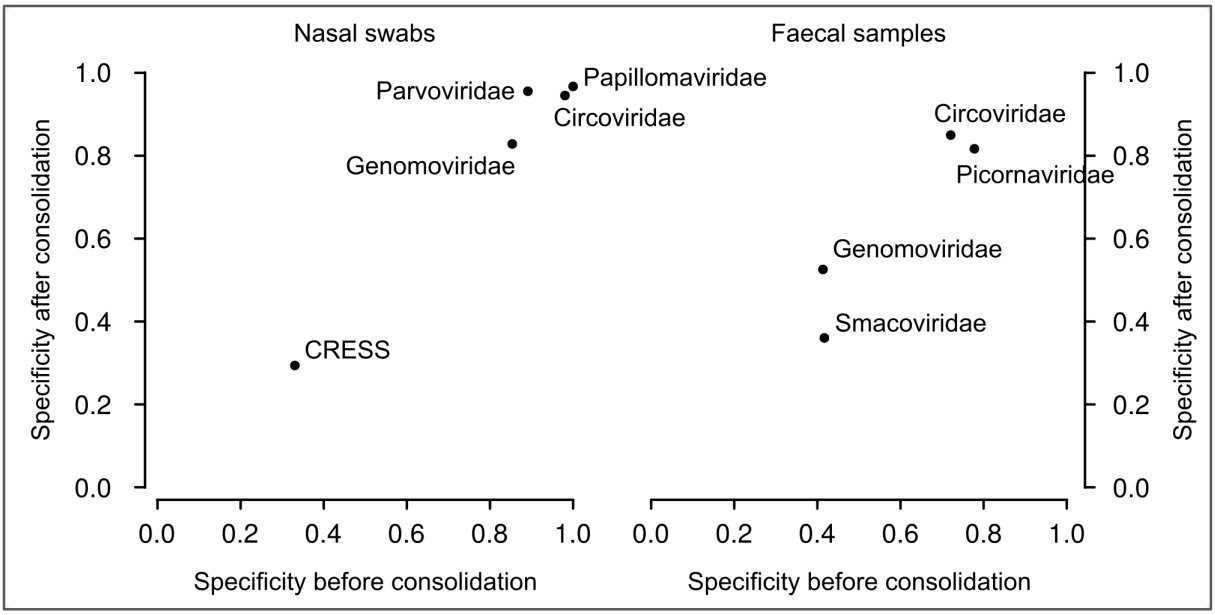


Figure 17: Sample specificity for viral families
The sample specificity for the viral families with enough read counts to be included is shown. The value 0.0 means the viral family is present in all samples, 1.0 means the family is very sample specific.
This figure was generated by MWSchmid GmbH.

4 Discussion

There is very little known about the bacteriome and virome of healthy Swiss dairy cows. Up to the time of writing there are no studies published exploring the nasal or faecal microbiome of cows in Switzerland. To fill that gap, we want to describe the composition of the bacteriome and virome in nasal swabs and faecal samples of healthy Swiss dairy cows. The study was conducted in the context of an extended health examination upon consolidation of three dairy cow herds into one herd in a newly built stable. The union of these herds posed an excellent and rare opportunity to study the effects of intermingling cow herds on the composition of the microbiome. The aim was to explore if there is a pattern of bacterial and viral transmission apparent. Nasal swabs and faecal samples of all individuals of the three cow herds were taken before and after mixing of the herds. The sample types, nasal swabs and faecal samples, were chosen to give a good insight into two important organ systems. Respiratory diseases play an important role in bovine health, causing massive economic losses and impairing animal health and welfare in young livestock and beef cattle. A sound digestive system naturally is crucial for a cow to stay healthy and being able to produce good quality milk and have a successful reproduction cycle. The samples were analysed using a metagenomic approach as it allows to detect any nucleic acid present in a sample. The microbiome of each herd was analysed comparing the bacteriome and virome from before to after the union of the herds.

4.1 Bacteriome

In several studies the most common phyla described in the nasal cavity and the bovine nasopharynx were *Proteobacteria*, *Tenericutes, Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Gaeta et al., 2017; Holman et al., 2017; Nicola et al., 2017; Timsit et al., 2016). In the samples of this study all of the above-mentioned phyla were frequently detected apart from *Tenericutes*, which we have only seen on a low level as described in chapter *3.1.1*. The most relevant genus in this phylum is *Mycoplasma* (Brown, 2019). *Mycoplasma*, especially *Mycoplasma bovis*, is a pathogen of the bovine respiratory disease complex (Griffin et al., 2010). The lack of *Tenericutes* respectively *Mycoplasma* could be explained by the fact that the animals of this study were adult and healthy. In most studies on the nasal bacteriome young cattle, around the time of moving to the feedlot, was examined. This is the age group which is most prone to develop bovine respiratory disease (BRD), therefore BRD pathogens are expected to be less prevalent in adult cows.

In the faecal samples of this study the majority of reads were assigned to *Firmicutes* and *Bacteroidetes*. This result is in line with the findings of other studies examining the bovine

faecal bacteriome, although depending on the outline of the studies, the reported ratio of these two phyla is varying (Durso et al., 2010; Kim et al., 2014; McGarvey et al., 2010; Ozutsumi et al., 2005; Shanks et al., 2011; Tang et al., 2017). It seems that these two phyla represent the core faecal bacteriome of cows.

In the nasal swabs eight bacterial families, *Enterobacteriaceae (Proteobacteria)*, *Lachnospiraceae (Firmicutes)*, *Lactobacillaceae (Firmicutes)*, *Moraxellaceae (Proteobacteria)*, *Rhizobiaceae (Proteobacteria)*, *Ruminococcaceae (Firmicutes)*, *Sphingomonadaceae (Proteobacteria)* and *Staphylococcaceae (Firmicutes)* were identified as a typical bacteriome composition before and after consolidation, as shown in *Figure 3A*. All families mentioned above, apart from *Rhizobiaceae*, have previously been detected in the upper respiratory tract of calves and beef cattle (Gaeta et al., 2017; Holman et al., 2015a; Holman et al., 2017; Nicola et al., 2017; Timsit et al., 2016). *Rhizobiaceae* are nitrogen fixating bacteria previously found in cow manure (Wong et al., 2016), possibly ending up in the nasal cavity via contamination through faeces or feed. *Pasteurellaceae,* a well-represented bacterial family in the bovine upper respiratory tract in other studies (Gaeta et al., 2017; Nicola et al., 2017; Timsit et al., 2016), was not frequently detected in the study at hand. The same eight families were present in all the representative nasal swab samples, but the ratio slightly changed upon consolidation of the herds. The changes in proportion were different for each pre-sampling farm. The nasal bacteriome of healthy adult cows, once established, seems to only shift within a certain range upon changes in the environment.

The composition of the representative faecal samples of the pre-sampling farms was more uniform than the nasal bacteriome, as shown in *Figure 3B*. This indicates that the ratio of bacterial families present in the faeces is more constant than in the nasal bacteriome. The prominent families in the faecal bacteriome were *Verrucomicrobiaceae (Verrucomicrobia)*, *Bacteroidaceae (Bacteroidetes)*, *Lachnospiraceae (Firmicutes)*, *Porphyromonadaceae (Bacteroidetes)*, *Rikenellaceae (Bacteroidetes)* and *Ruminococcaceae (Firmicutes)*. These are all families commonly present in bovine faecal manure (Dowd et al., 2008; Durso et al., 2010; Kim et al., 2014; McGarvey et al., 2010; Ozutsumi et al., 2005; Shanks et al., 2011; Tang et al., 2017). *Prevotellaceae* were also frequently found in bovine faeces in other studies (Dowd et al., 2008; Durso et al., 2010; Kim et al., 2014; Shanks et al., 2011) but were not among the most abundant families in the present study. Studies have shown that *Prevotellaceae* are highly influenced by feed (Durso et al., 2012; Rice et al., 2012) and rarely detected in the faecal bacteriome of cattle fed with silage (Kim et al., 2014). This could be the reason *Prevotellaceae* were only scarcely detected in the present study as the feed of two thirds of the Swiss dairy

population contains silage (Bundesamt für Landwirtschaft BLW, 2020). We did not collect any data on the feed of the cows examined in this study, but most likely their diet also contained silage to a certain extend.

In the nasal cavity the relative abundance of *Cardiobacteriaceae*, *Enterobacteriaceae*, *Lactobacillaceae, Methylobacteriaceae*, *Moraxellaceae* and *Prevotellaceae* changed strongest upon mixing of the herds, as shown in *Figure 4A*. The *Cardiobacteriaceae* reads could be assigned to the genus *Suttonella* or remained unknown. Bacteria of the genus *Suttonella* have previously been found in the ruminal flora of dairy heifers (Zhan et al., 2017), so the finding of Suttonella reads in nasal swab material could suggest a contamination with rumen content. *Enterobacteriacea* are a bacterial family of the intestinal flora (Selbitz and Alber, 2011). Apart from many reads which could not be assigned to a genus, reads of the genera *Buttiauxella*, *Obesumbacterium*, *Pantoea* and *Serratia* were detected. *Buttiauxella* is a bacterial genus that can be found in faeces of wild ruminants (Lauková et al., 2018), in beef (Ercolini et al., 2009; Säde et al., 2013) and pasteurized milk (He et al., 2009). *Obesumbacterium* is a genus rarely found. It is a known brewery contaminant and grows in beer wort alongside yeasts (Brenner et al., 2005). *Pantoea* has previously been found in milk samples of cows (Martins et al., 2018; Savage et al., 2017). Bacteria of the genus *Serratia* can be found in beef (Ercolini et al., 2009; Säde et al., 2013) and bovine milk (Hagi et al., 2013; Martins et al., 2018; Savage et al., 2017). The *Enterobacteriaceae* found in nasal swabs are most likely faecal contamination, as there are no studies published which would suggest they are part of the nasal bacteriome. *Lactobacillaceae* are a part of the healthy nasal bacteriome (Holman et al., 2015a) and also of the rumen microbiome (Matthews et al., 2019). *Methylobacteriaceae* are a known part of the flora in bovine milk (Andrews et al., 2019; Taponen et al., 2019) but have also been identified as laboratory contaminants in microbiome studies (Salter et al., 2014), which could indicate that their finding in nasal swabs is the result of contamination. *Moraxellaceae* are a well-known part of the bovine nasal bacteriome (Gaeta et al., 2017; Holman et al., 2017; Lima et al., 2016; Nicola et al., 2017; Timsit et al., 2016). *Prevotellaceae* are part of the bovine microbiome in several organs. Bacteria of this family can be found in the upper respiratory tract (Lima et al., 2016), in the gastro-intestinal tract (de Oliveira et al., 2013; Dowd et al., 2008; Henderson et al., 2015; Mao et al., 2015; Matthews et al., 2019) and also in the bovine uterus (Ault et al., 2019a; Galvão et al., 2019). Overall, we found the major components of the normal flora of the bovine nasopharyngeal cavity and in addition possible contaminants from the gastro-intestinal tract. The cows we sampled were housed in an open plan shed where they could roam freely. It

would be interesting to analyse if different housing systems or keeping animals on pastures only influences the composition of the nasal bacteriome and particularly the degree of faecal contamination.

In the faecal samples (*Figure 4B*) the relative abundance of *Bifidobacteriaceae* and *Ruminococcaceae* increased the most, the abundance of *Verrucomicrobiacea*e decreased the most upon consolidation. These bacterial families are all part of the healthy bovine faecal microbiome (de Oliveira et al., 2013; Kim et al., 2014; Mao et al., 2015; Tang et al., 2017).

Interestingly, in nasal swabs clusters of samples showed similar changes in occurrence for most OTUs upon consolidation *(Figure 5A)*. However, it was not possible to identify a grouping factor which may drive these changes in bacteriome composition. These sample clusters were neither associated to the main farms *(Figure 5B)* or the three pre-sampling farms *(Figure 5C)* nor to any other factor from the animals' history we analysed, such as farm of birth, communal grazing on alps, age, animal hospital stays or cattle market visits. The clusters of similar changes did also not correspond to the composition of the bacteriome before consolidation. Therefore, we assume that changes of the nasal bacteriome depend more on individual host-related factors rather than environmental factors. These host-related factors may be genetic background, general health status, status of the immune system or most likely a combination of several factors. It is not possible to draw a conclusion regarding the influence of the genetic background from this study, as animals with different genetic background were pooled together. No detailed health data was obtained nor was any examination regarding the immune system conducted. On long term, i.e., two years, the united herd didn't show major infectious problems, neither by viruses nor by bacteria. The only major bacterial problem found was digital dermatitis by Mortellaro's disease.

Overall, in the faecal samples there were similar changes (increase and decrease of the same OTUs) in bacteriome composition observed for most of the samples upon consolidation, which can be seen in the horizontal pattern of the heatmaps of *Figure 5*. There seems to be one or several factors influencing most of the samples in a similar way. This shift could possibly be explained by the change in feed. It has previously been suggested that general management, especially feeding management, influences the gut microbiota (Shanks et al., 2011; Tang et al., 2017). Other studies suggest that yearly fluctuations in circulating bacteria (Rudi et al., 2012) or unknown animal-to-animal differences (Durso et al., 2010) are the main factors influencing the microbiome composition. These data suggest that the gut bacteriome composition is influenced by many factors, and different study results might be due to different study designs.

Looking at the changes upon consolidation in more detail, significantly more reads of *Staphylococcaceae, Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillaceae* and *Moraxellaceae* and significantly less reads of *Enterobacteriaceae* and unknown bacterial family were detected after consolidation in nasal swabs *(Figure 6)*. All these bacterial families are to be expected in the upper respiratory tract (Gaeta et al., 2017; Holman et al., 2015a; Holman et al., 2017; Lima et al., 2016; Nicola et al., 2017; Timsit et al., 2016).
In the faecal samples the abundance of five bacterial families of the healthy bovine gastro-intestinal bacteriome (Alipour et al., 2018; Chaucheyras-Durand and Ossa, 2014; de Oliveira et al., 2013; Dowd et al., 2008; Durso et al., 2010; Kim et al., 2014; Mao et al., 2015; McGarvey et al., 2010; Shanks et al., 2011; Tang et al., 2017; Zaheer et al., 2019) significantly changed with the union of the herds. *Methanobacteriaceae*, *Lachnospiraceae* and *Bifidobacteriaceae* significantly increased upon consolidation, *Verrucomicrobiaceae* and *Bacteroidaceae* significantly decreased *(Figure 6)*.

Furthermore, in nasal swabs there seem to be a lot of sample specific OTUs before and after consolidation *(Figure 7)*. Therefore, it seems that the nasal microbiome is rather sample specific, and its composition changes rather individually upon intermingling of the herds. Regarding the sample specificity in faecal samples *(Figure 7)*, most OTUs were common among all samples sequenced before and after consolidation. There were no OTUs, neither in nasal swabs nor in faecal samples, which spread from a few animals to a lot or the other way around upon intermingling of the herds.

Considering all calculated diversity indices *(Table 6)*, the bacterial diversity decreased in the nasal cavity upon mixing of the herds. However, the changes in nasal swabs were only significant for the effective richness but not for the species richness, biodiversity and Pielou's evenness. The bacterial community composition (Manhattan distance) changed significantly within a herd upon consolidation. But comparing the different nasal swabs pre consolidation to each other and post consolidation to each other, there were no significant differences in bacteriome composition. Overall, the sample influenced the change in the nasal microbiome stronger in all calculated factors than the herd consolidation. This indicates that it is hard to predict what kind of changes the nasal bacteriome undergoes upon moving a cow into a new environment.
Regarding the diversity of the faecal samples, there was a not significant increase in species richness, but in all the other diversity indices there was a significant decrease of diversity

observed *(Table 6)*. It has to be noted that species richness does only consider the number of different species but not their abundance. It is therefore not a very precise diversity measurement. Considering this, we can conclude that upon consolidation of the herds there is a loss of diversity in the faecal samples. The bacterial community structure (Manhattan distance) was significantly different between different samples before and after consolidation. But there were also significant changes upon consolidation within the same main farm. Generally, the faecal bacteriome became more uniform upon consolidation, but the differences between the samples were always larger than the differences between the two sampling points, similar to what we already observed for the nasal microbiome. This shows that, also in faeces, individual animal factors seem to have the stronger influence on the bacterial diversity than external factors, like mixing herds and changing the farm as in our study. This has already previously been reported (Durso et al., 2010; Rudi et al., 2012). The study of *Durso et al.* showed that the variation in the microbiome composition of beef cattle could not be explained by breed, gender, age or environmental factors, like weather of feed. *Rudi et al.* found a significant influence of the sampling year on the bacterial abundance. Nevertheless, the consolidation in our study also had a common influence on the faecal microbiome, as the microbiome structure changed significantly and became more similar, which could not be observed in the nasal microbiome.

In conclusion, the changes of diversity in the faecal bacteriome upon consolidation were stronger and more uniform than the ones in the nasal bacteriome, indicating that the faecal bacteriome is more readily influenced by environmental changes. The nasal bacteriome in contrast is generally more varied to start with, and the way it changes upon mixing the herds could not be accounted to a specific factor. In both cases, nasal and faecal bacteriome, differences between individual samples (i.e., sample pools) were larger than changes upon herd consolidation.

4.2 Virome

In nasal swabs the most frequently detected viruses, present in over 90% of the samples, belong to the group of not assigned CRESS DNA viruses (not (yet) assigned to a viral family). In approximately a third to half of the samples reads of the *Genomoviridae* and in around a fifth reads of *Parvoviridae* were detected. Globally important epizootic viruses that may be found in nasal swabs, such as bovine herpesvirus 1 (*Herpesviridae*) and bovine viral diarrhoea virus (*Flaviviridae*) (Fulton et al., 2016; Mitra et al., 2016; Moore et al., 2015), have not been detected in this study. These results were expected as Switzerland is free of bovine herpesvirus 1 and

nearly free of bovine viral diarrhoea virus (Federal Food Safety and Veterinary Office, 2018). In the faecal virome the most prevalent families were *Genomoviridae* and *Smacoviridae*, detected in up to 68% and 84% of the samples, followed by *Circoviridae* and *Picornaviridae* in approximately a quarter to a third of the samples *(Figure 16)*. Apart from the not assigned CRESS DNA viruses, also *Circoviridae*, *Genomoviridae* and *Smacoviridae* belong to the group of CRESS DNA viruses *(Table 7)*.

According to the International Committee on Taxonomy of Viruses (ICTV) the phylum *Cressdnaviricota* contains seven families: *Bacilladnaviridae*, *Circoviridae*, *Geminiviridae, Genomoviridae, Nanoviridae*, *Redondoviridae, Smacoviridae* (Krupovic et al., 2020). They have a small circular ssDNA genome up to 6 kilobases long and encode for at least two proteins, a replication-associated protein (Rep) and a capsid protein (Díez-Villaseñor and Rodriguez-Valera, 2019; Rosario et al., 2012). For replication they seem to use rolling circular replication and depend on cellular proteins (Rosario et al., 2012). Recently, with more and more metagenomic studies being performed, an increasing amount of new ssDNA virus sequences are detected in various environments and hosts (Zhao et al., 2019). *Bacilladnaviridae* have been associated with algae, *Geminiviridae* and *Nanoviridae* with plants, *Genomoviridae* with fungi and insects (Krupovic et al., 2020). Hosts of *Circoviridae* are a variety of mammals, birds and fish (Breitbart et al., 2017). *Circoviridae* include known pathogens of livestock, like porcine circovirus *(Rosario et al., 2012; Saikumar and Das, 2019)*. *Redondoviridae* have been found in the human respiratory tract (Abbas et al., 2019) and *Smacoviridae* in faecal matter of various animals and humans (Varsani and Krupovic, 2018).

In *Table 8* the viruses found in this study which belong to the phylum *Cressdnaviricota* are shown. *Circoviridae* were more prevalent and more reads were detected in faecal samples than in nasal swabs. Viruses closely related to the dromedary stool-associated circular ssDNA virus were most frequently found in this family. This virus was first sequenced in faeces of mainly healthy dromedary camels in Dubai (Woo et al., 2014). A closely related virus, bovine faeces associated circular DNA virus, has already previously been detected in bovine faeces (Steel et al., 2016). Most reads of the family *Genomoviridae* in nasal swabs were most closely related to faecal-associated gemycircularvirus 6 and reads from faeces to Alces alces faeces associated microvirus MP11 5517. Faecal-associated gemycircularvirus 6 was first detected in faecal matter of a bird (Chatham Island Warbler) (Sikorski et al., 2013). Subsequently, gemycircularviruses have also been detected in cow manure (Steel et al., 2016). However, these are not closely related to the gemycircularviruses detected in our study. Alces alces faeces associated microvirus MP11 5517 was first sequenced from freshly deposited faeces of a wild

moose (Kraberger et al., 2018). The analysis tool used in this study (https://fgcz-sushi.uzh.ch) assigned this virus to the family of *Genomoviridae*. In literature though it is assigned to the family of *Microviridae* (Kraberger et al., 2018). The viruses of this family are bacteriophages found in a variety of environments, for example water samples or stool samples (Roux et al., 2012). The genus *Microvirus* is composed of *Enterobacteria* phages (Roux et al., 2012), which could explain the finding in faecal samples. Reads most closely related to bovine associated bovismacovirus 1 were detected in nasal swabs and faecal samples. In faeces it was the most frequent virus with reads detected in 68% of the samples pre and 84% of the samples post consolidation. This virus was first detected in febrile and anorexic calves in Korea in 2011. Subsequently, in the same study the virus was found in approximately 60% of faecal samples from healthy adult cows but not in the feed of the respective cows. No conclusive statement could be made regarding a potential pathogenicity, as in the sick calves also reads of pathogenic bacteria were found (Kim et al., 2012). Smacoviruses have been found in faeces of various animals (Steel et al., 2016) and in human stool (Ng et al., 2015b), but it is not yet known if these species are really the hosts of these viruses (Díez-Villaseñor and Rodriguez-Valera, 2019). Recent CRISPR analysis has indicated that smacoviruses might actually infect archeae instead of eukaryotes (Díez-Villaseñor and Rodriguez-Valera, 2019). In most nasal swabs CRESS DNA viruses, which are not assigned to a viral family (not assigned CRESS DNA viruses), were detected but only in a few faecal samples. The sequences of "uncultured virus" found in over 90% of the nasal swabs were first detected in influent sewage of a wastewater treatment plant in Florida (Pearson et al., 2016) and the "Cress virus sp." in tissue of various fish and packaged beef (Tisza et al., 2020).

To our knowledge there are no published studies examining the nasal or faecal virome of dairy cows. Nevertheless, the results of studies researching the virome of feedlot cattle seem to be similar to the findings of the viral spectrum in this study. It has been shown that reads of CRESS DNA viruses are found in nasal swabs of cattle, both in healthy individuals and animals showing symptoms of bovine respiratory disease (BRD) (Mitra et al., 2016), and *Genomoviridae*, *Smacoviridae* and also not assigned CRESS DNA viruses have been found prevalent in faeces of several cows in New Zealand (Steel et al., 2016).

Apart from CRESS DNA viruses, also *Parvoviridae* and *Picornaviridae* were frequently detected in this study, with *Parvoviridae* mainly present in nasal swabs and *Picornaviridae* in faecal samples *(Table 9)*.

Parvoviridae are small, mostly host or tissue specific viruses with a single-stranded genome of 4-6 kilobases (Cotmore et al., 2019). They are the only single-stranded DNA viruses which have a linear genome (Rosario et al., 2012). We found mainly members of the genus *Bocaparvovirus* and a few reads of *Dependoparvovirus* in our samples *(Table 7)*. These two genera belong to the subfamily of Parvovirinae, which infect vertebrates (Cotmore et al., 2019). Previously, it has been found that Parvoviridae are prevalent in the respiratory tract of cattle (Zhang et al., 2019a). The most reads in our study were closely related to ungulate bocaparvovirus 6 in nasal swabs, a *Bocaparvovirus* and the only fully sequenced genome in this study. This virus was first described in nasal swabs of feedlot cattle in the USA and Mexico (Mitra et al., 2016). The study of Mitra et al. compared the nasal virome of feedlot cattle with bovine respiratory disease (BRD) and asymptomatic pen-mates in the USA and Mexico. Ungulate bocaparvovirus 6 was equally frequently detected in asymptomatic and BRD symptomatic animals, but it is not yet conclusively known if this virus has an impact on the health status (Mitra et al., 2016). Mitra et al. detected more reads of the following viruses in healthy compared to diseased animals: enterovirus E and F *(Picornaviridae)*, ungulate bocaparvovirus 6 *(Parvoviridae)*, bovine nidovirus *(Tobaniviridae)*, bovine adenovirus 3 *(Adenoviridae)*, bovine adeno-associated virus *(Parvoviridae) (Mitra et al., 2016)*. Of these mentioned viruses, we found in the present study ungulate bocaparvovirus 6 in 12 (15%) and adeno-associated virus in 2 out of 78 (3%) nasal swab samples. Reads of ungulate bocaparvovirus 6 have also been found in a Canadian study, where the nasal and tracheal virome of healthy cattle and cattle with bovine respiratory disease (BRD) was compared (Zhang et al., 2019a). In the study of Zhang et al. a lower number of total viral reads was found in healthy animals than in diseased, but interestingly, out of the 21 detected viruses four, bovine nidovirus *(Tobaniviridae)*, influenza C virus *(Orthomyxoviridae)*, ungulate bocaparvovirus 6 *(Parvoviridae)* and WU polyomavirus *(Polyomaviridae)*, occurred more often in healthy than in animals with BRD (Zhang et al., 2019a). Bovine nidovirus, influenza C virus and ungulate bocaparvovirus 6 are known viruses found in cattle (Mitra et al., 2016; Tokarz et al., 2015; Zhang et al., 2018). WU polyomavirus is a virus found in humans but has also previously been found in aerosol samples of a slaughter house, specifically in the cattle processing area but not in the sheep processing area (Hall et al., 2013). In the present study only reads of ungulate bocaparvovirus 6 were found, none of the others.

In faeces ungulate bocaparvovirus 1, previously known as bovine parvovirus 1 (https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=20132565&src=NCBI&ictv_id=20132565, July 26, 2020), was the only

member of *Parvoviridae* detected. It was only sequenced in 2 out of 74 (3%) faecal samples. Ungulate bocaparvovirus 1 has first been detected in calves with diarrhoea (Abinanti and Warfield, 1961). Apart from enteric disease in calves, it has also been associated with reproductive disorders in cows (Sandals et al., 1995). Bovine parvovirus was first detected in Switzerland in 1988, in a study investing the prevalence of bovine parvovirus in Switzerland and its possible role in causing abortion. Bovine parvovirus didn't proof to be a major virus causing abortion in Switzerland (Hässig et al., 1988). In dairy cattle a high seroprevalence has been shown with up to 70% of the cows having antibodies against bovine parvovirus, but clinical diseases in adults are rare (Manteufel and Truyen, 2008). According to the ICTV ungulate bocaparvovirus 1 and 6 are the only member of the genus *Bocaparvovirus* associated with cattle (https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae/1041/genus-bocaparvovirus, July 26, 2020), and both were detected in this study.

In faecal samples mainly CRESS DNA viruses and *Picornaviridae* sequences were detected. Most of the *Picornaviridae* reads were assigned to the genus *Kobuvirus (Table 7)*. They were detected in 17 out of 74 samples (23 %). Kobuvirus is a small virus with a positive sensed single-stranded RNA genome of around 8 kilobases (Reuter et al., 2011). In 2003, a group in Japan sequenced for the first-time cattle associated kobuvirus reads out of faeces of healthy cows. In 16.7% of their tested faecal samples reads for bovine kobuvirus were detected (Yamashita et al., 2003). The kobuvirus reads found in the present study were most closely related to the species *Aichivirus B* (bovine kobuvirus) and *D* (kagovirus 1) (https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/686/genus-kobuvirus, December 16, 2019). Bovine kobuvirus has been detected worldwide in faeces of healthy (Barry et al., 2011; Di Martino et al., 2012; Jeoung et al., 2011; Reuter and Egyed, 2009; Yamashita et al., 2003) and diarrheic cattle (Di Martino et al., 2012; Jeoung et al., 2011; Khamrin et al., 2008; Mauroy et al., 2009; Mohamed et al., 2018; Otomaru et al., 2016; Park et al., 2011; Ribeiro et al., 2014; Ribeiro et al., 2017). It is yet not clear if there is a correlation between diarrhoea and kobuvirus present in the intestine. Calves seem to be more often positive than adult cows (Jeoung et al., 2011; Park et al., 2011; Reuter and Egyed, 2009; Ribeiro et al., 2014). In the present study we only tested adult cows, therefore we cannot make any statement regarding the prevalence of kobuvirus in calves of the respective farms. In pigs a similar situation is observed in studies. Kobuvirus is often detected, especially in young piglets. But no clear association with disease could be made

so far, as it is also regularly found in healthy pigs (Jackova et al., 2017; Nantel-Fortier et al., 2019).

The representative virome samples *(Figure 13)* show a relatively limited viral diversity in nasal swabs and faecal samples. Upon mixing of the herds, a slight loss of diversity seems to have occurred. The same was observed in the bacteriome. Regarding the changes in viral read counts of the individual farms upon consolidation, relatively few changes were observed. However, changes in the faecal virome seem larger than in the nasal virome *(Figure 14, Figure 15)*. Also, regarding the prevalence of viral families, the nasal samples showed only few changes upon consolidation. In faecal samples though, significant changes in prevalence of *Circoviridae* and *Smacoviridae* were observed *(Figure 16)*. *Circoviridae* were detected in significantly less and *Smacoviridae* in significantly more faecal samples after consolidation. These findings indicate, similar to the findings in the bacteriome, that the nasal virome is quite robust once established, and the faecal virome is more influenced by environmental changes. To the authors knowledge there are no studies available regarding the influence of feed on the faecal virome. Nevertheless, it could be imagined that the change in feed also influences the faecal virome, similar as seen in the faecal bacteriome, especially as this has been shown in the ruminal virome (Anderson et al., 2017).

In addition to the pooled samples, the virome of some individual samples was sequenced. The twin cows showed a fairly similar nasal and faecal virome to each other before and after consolidation and less similar to their respective pool *(Figure 11)*. These two cows seemed to have a relatively robust and closely related virome. This could be explained by their blood relationship or also their similar upbringing and farm history. With the other individually tested samples, the aim was to detect a potential loss of sensitivity of virus detection upon pooling. In the nasal samples of the two cows the diversity seems to decrease upon pooling, pointing to a loss of sensitivity. In the two individually sequenced faecal samples the opposite was observed, as the diversity was increased in the respective pools *(Figure 12)*. The decrease in diversity in nasal swabs could possibly be explained by a dilution effect. The pooling could result in the inability of the metagenomic approach to detect viruses with very few read count. In the majority of nasal swab pools, and also in these individually sequenced ones, most reads were assigned to not assigned CRESS DNA viruses. This group of viruses is highly diverse, and therefore a loss of certain species upon pooling is quite likely. In faeces less different viruses were detected, and therefore the reads count most likely adds up upon pooling. However, the

number of individually sequenced samples was very small, and therefore we cannot generalize these results.

Summarizing, in both sample types the most frequent viruses detected were single-stranded DNA viruses. The diversity in the bovine nasal and faecal virome was rather small, and upon intermingling of the herds, a slight loss in diversity could be seen. Only few changes in virome composition occurred upon herd consolidation. The larger changes were observed in the faecal virome, therefore the nasal virome seems more robust than the faecal virome.

4.3 Limitations

The method of metagenomic approach used in this study has knowingly a lower sensitivity than conventional methods, like qPCR (Zhang et al., 2019b). On the other hand, Next Generation Sequencing can detect all genomic material present in a sample, and therefore new or unexpected bacteria or viruses might be detected. Due to the large number of individual samples and limited time, we pooled up to six samples for sequencing, hence we cannot draw conclusions on the microbiome of individual animals, and a loss of species with very few reads was expected. Further, it should be stated that the virome found in nasal swabs may differ from the viral reads found in tracheal washes (Zhang et al., 2019a). Consequently, we cannot draw conclusions on the whole respiratory virome from just looking at the nasal virome. Nevertheless, analysing nasal swabs in order to get an insight into the respiratory virome is the most practical and least invasive way and allows sampling of larger numbers of animals. In addition, these viruses may be shed and exchanged more readily than the ones in deeper sections of the respiratory tract. Therefore, they are particularly interesting when studying the dynamics of the microbiome upon herd consolidations. However, it could be shown that the bacteriome sequenced from nasal swabs is very similar to the bacteriome sequenced from deep nasopharyngeal swabs (McDaneld et al., 2018). For the sequencing runs no positive or negative controls were used, as each additional sample reduces the output per sample. Consequently, the risk of a potential contamination was taken into consideration when performing the analysis. Another limitation was the fact that animals may have been moved between herds prior to consolidation, or single cows, that were not sampled before, newly brought into the new herd. Cattle movements and trade is very active in Switzerland, and it was impossible to avoid these scenarios. However, the majority of the animals was sampled twice according to the scheme *(Table 1)*.

4.4 Conclusions

We used a metagenomic approach to investigate the nasal and faecal bacteriome and virome of three independent dairy herds, before and after they were intermingled in a new stable as one herd.

The composition of the nasal bacteriome showed a higher variability than the faecal bacteriome, which was in the general structure quite uniform. The nasal and faecal bacteriome both changed upon consolidation. The nasal bacteriome showed stronger changes in read count and therefore in the ratio of the bacterial families. The changes seem to be driven by samples specific factors, which could not be identified in this study. In the faecal bacteriome significant changes in bacterial diversity were observed. The samples became more similar to each other upon union of the herds, possibly explained by the change of feed. But in faeces also a strong influence of sample specific factors was shown.

In the virome less reads and less different species, compared to the bacteriome, were detected. In both samples types a lot of CRESS DNA viruses were found. There is still a lot unknown about these viruses, like host species or association with disease. No known pathogenic viruses were found, with exception of a few reads of bovine rhinitis virus. In both sample types a slight decrease in viral diversity was seen upon consolidation. The nasal virome showed less changes upon mixing of the herds than the faecal virome.

No bacteria or virus was observed to completely disappear, emerge or significantly spread upon mixing of the herds. Our data rather showed that the established microbiome of healthy adult cows seems to be quite stable, even in a significantly changing environment.

Overview of Figures and Tables

References

Abbas, A. A., L. J. Taylor, M. I. Dothard, J. S. Leiby, A. S. Fitzgerald, L. A. Khatib, R. G. Collman, and F. D. Bushman, 2019, Redondoviridae, a Family of Small, Circular DNA Viruses of the Human Oro-Respiratory Tract Associated with Periodontitis and Critical Illness: Cell Host Microbe, v. 26, p. 297.

Abinanti, F. R., and M. S. Warfield, 1961, Recovery of a hemadsorbing virus (HADEN) from the gastrointestinal tract of calves: Virology, v. 14, p. 288-9.

Addis, M. F., A. Tanca, S. Uzzau, G. Oikonomou, R. C. Bicalho, and P. Moroni, 2016, The bovine milk microbiota: insights and perspectives from -omics studies: Mol Biosyst, v. 12, p. 2359-72.

Aggarwal, C. C., A. Hinneburg, and D. A. Keim, 2001, On the Surprising Behavior of Distance Metrics in High Dimensional Space, p. 420-434.

Alipour, M. J., J. Jalanka, T. Pessa-Morikawa, T. Kokkonen, R. Satokari, U. Hynönen, A. Iivanainen, and M. Niku, 2018, The composition of the perinatal intestinal microbiota in cattle: Sci Rep, v. 8, p. 10437.

Anderson, C. L., M. B. Sullivan, and S. C. Fernando, 2017, Dietary energy drives the dynamic response of bovine rumen viral communities: Microbiome, v. 5, p. 155.

Andrews, T., D. A. Neher, T. R. Weicht, and J. W. Barlow, 2019, Mammary microbiome of lactating organic dairy cows varies by time, tissue site, and infection status: PLoS One, v. 14, p. e0225001.

Ault, T. B., B. A. Clemmons, S. T. Reese, F. G. Dantas, G. A. Franco, T. P. L. Smith, J. L. Edwards, P. R. Myer, and K. G. Pohler, 2019a, Bacterial taxonomic composition of the postpartum cow uterus and vagina prior to artificial insemination1: J Anim Sci, v. 97, p. 4305-4313.

Ault, T. B., B. A. Clemmons, S. T. Reese, F. G. Dantas, G. A. Franco, T. P. L. Smith, J. L. Edwards, P. R. Myer, and K. G. Pohler, 2019b, Uterine and vaginal bacterial community diversity prior to artificial insemination between pregnant and nonpregnant postpartum cows1: J Anim Sci, v. 97, p. 4298-4304.

Baechlein, C., N. Fischer, A. Grundhoff, M. Alawi, D. Indenbirken, A. Postel, A. L. Baron, J. Offinger, K. Becker, A. Beineke, J. Rehage, and P. Becher, 2015, Identification of a Novel Hepacivirus in Domestic Cattle from Germany: J Virol, v. 89, p. 7007-15.

Barry, A. F., J. Ribeiro, A. F. Alfieri, W. H. van der Poel, and A. A. Alfieri, 2011, First detection of kobuvirus in farm animals in Brazil and the Netherlands: Infect Genet Evol, v. 11, p. 1811-4.

Bauermann, F. V., L. R. Joshi, K. A. Mohr, G. F. Kutish, P. Meier, C. Chase, J. Christopher-Hennings, and D. G. Diel, 2017, A novel bovine papillomavirus type in the genus Dyokappapapillomavirus: Arch Virol, v. 162, p. 3225-3228.

Benjamini, Y., and Y. Hochberg, 1995, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing: Journal of the Royal Statistical Society. Series B (Methodological), v. 57, p. 289-300.

Berg Miller, M. E., C. J. Yeoman, N. Chia, S. G. Tringe, F. E. Angly, R. A. Edwards, H. J. Flint, R. Lamed, E. A. Bayer, and B. A. White, 2012, Phage-bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome: Environ Microbiol, v. 14, p. 207-27.

Bhatt, V. D., V. B. Ahir, P. G. Koringa, S. J. Jakhesara, D. N. Rank, D. S. Nauriyal, A. P. Kunjadia, and C. G. Joshi, 2012, Milk microbiome signatures of subclinical mastitis-affected cattle analysed by shotgun sequencing: J Appl Microbiol, v. 112, p. 639-50.

Bicalho, M. L. S., T. Santin, M. X. Rodrigues, C. E. Marques, S. F. Lima, and R. C. Bicalho, 2017, Dynamics of the microbiota found in the vaginas of dairy cows during the

transition period: Associations with uterine diseases and reproductive outcome: J Dairy Sci, v. 100, p. 3043-3058.
Blomström, A. L., V. Oma, M. Khatri, H. H. Hansen, M. Stokstad, M. Berg, and M. Myrmel, 2017, Genome Sequence of a Bovine Rhinitis B Virus Identified in Cattle in Sweden: Genome Announc, v. 5.
Bolger, A. M., M. Lohse, and B. Usadel, 2014, Trimmomatic: a flexible trimmer for Illumina sequence data: Bioinformatics, v. 30, p. 2114-20.
Bonsaglia, E. C. R., M. S. Gomes, I. F. Canisso, Z. Zhou, S. F. Lima, V. L. M. Rall, G. Oikonomou, R. C. Bicalho, and F. S. Lima, 2017, Milk microbiome and bacterial load following dry cow therapy without antibiotics in dairy cows with healthy mammary gland: Sci Rep, v. 7, p. 8067.
Bouzalas, I. G., D. Wüthrich, J. Walland, C. Drögemüller, A. Zurbriggen, M. Vandevelde, A. Oevermann, R. Bruggmann, and T. Seuberlich, 2014, Neurotropic astrovirus in cattle with nonsuppurative encephalitis in Europe: J Clin Microbiol, v. 52, p. 3318-24.
Braslavsky, I., B. Hebert, E. Kartalov, and S. R. Quake, 2003, Sequence information can be obtained from single DNA molecules: Proc Natl Acad Sci U S A, v. 100, p. 3960-4.
Braun, U., M. Schönmann, F. Ehrensperger, M. Hilbe, D. Brunner, K. D. Stärk, and T. Giger, 1998, Epidemiology of bovine virus diarrhoea in cattle on communal alpine pastures in Switzerland: Zentralbl Veterinarmed A, v. 45, p. 445-52.
Breitbart, M., E. Delwart, K. Rosario, J. Segalés, A. Varsani, and Ictv Report Consortium, 2017, ICTV Virus Taxonomy Profile: Circoviridae: J Gen Virol, v. 98, p. 1997-1998.
Brenner, D., N. Krieg, J. Staley, G. Garrity, D. Boone, P. De Vos, M. Goodfellow, F. Rainey, and K. Schleifer, 2005, Bergey's Manual® of Systematic Bacteriology: Volume Two The Proteobacteria Part B The Gammaproteobacteria.
Brown, D. R., 2019, Tenericutes, Bergey's Manual of Systematics of Archaea and Bacteria, p. 1-3.
Bundesamt für Landwirtschaft BLW, S. E., 2020, Strategie Tierzucht 2030, p. 35.
Bundesamt für Statistik BFS, S. E., 2019, Die Bevölkerung der Schweiz 2018.
Chaucheyras-Durand, F., and F. Ossa, 2014, REVIEW: The rumen microbiome: Composition, abundance, diversity, and new investigative tools: The Professional Animal Scientist, v. 30, p. 1-12.
Cibulski, S. P., T. F. Teixeira, H. F. Dos Santos, F. E. de Sales Lima, C. M. Scheffer, A. P. Varela, D. A. de Lima, C. Schmidt, F. Silveira, L. L. de Almeida, and P. M. Roehe, 2016, Ungulate copiparvovirus 1 (bovine parvovirus 2): characterization of a new genotype and associated viremia in different bovine age groups: Virus Genes, v. 52, p. 134-7.
Clemmons, B. A., S. T. Reese, F. G. Dantas, G. A. Franco, T. P. L. Smith, O. I. Adeyosoye, K. G. Pohler, and P. R. Myer, 2017, Vaginal and Uterine Bacterial Communities in Postpartum Lactating Cows: Front Microbiol, v. 8, p. 1047.
Cotmore, S. F., M. Agbandje-McKenna, M. Canuti, J. A. Chiorini, A. M. Eis-Hubinger, J. Hughes, M. Mietzsch, S. Modha, M. Ogliastro, J. J. Pénzes, D. J. Pintel, J. Qiu, M. Soderlund-Venermo, P. Tattersall, P. Tijssen, and Ictv Report Consortium, 2019, ICTV Virus Taxonomy Profile: Parvoviridae: J Gen Virol, v. 100, p. 367-368.
Cox-Foster, D. L., S. Conlan, E. C. Holmes, G. Palacios, J. D. Evans, N. A. Moran, P. L. Quan, T. Briese, M. Hornig, D. M. Geiser, V. Martinson, D. vanEngelsdorp, A. L. Kalkstein, A. Drysdale, J. Hui, J. Zhai, L. Cui, S. K. Hutchison, J. F. Simons, M. Egholm, J. S. Pettis, and W. I. Lipkin, 2007, A metagenomic survey of microbes in honey bee colony collapse disorder: Science, v. 318, p. 283-7.
Cremonesi, P., C. Ceccarani, G. Curone, M. Severgnini, C. Pollera, V. Bronzo, F. Riva, M. F. Addis, J. Filipe, M. Amadori, E. Trevisi, D. Vigo, P. Moroni, and B. Castiglioni, 2018,

Milk microbiome diversity and bacterial group prevalence in a comparison between healthy Holstein Friesian and Rendena cows: PLoS One, v. 13, p. e0205054.
de Oliveira, M. N., K. A. Jewell, F. S. Freitas, L. A. Benjamin, M. R. Tótola, A. C. Borges, C. A. Moraes, and G. Suen, 2013, Characterizing the microbiota across the gastrointestinal tract of a Brazilian Nelore steer: Vet Microbiol, v. 164, p. 307-14.
de Souza, W. M., T. Dennis, M. J. Fumagalli, J. Araujo, G. Sabino-Santos, F. G. M. Maia, G. O. Acrani, A. O. T. Carrasco, M. F. Romeiro, S. Modha, L. C. Vieira, T. Ometto, L. H. Queiroz, E. L. Durigon, M. R. T. Nunes, L. T. M. Figueiredo, and R. J. Gifford, 2018, Novel Parvoviruses from Wild and Domestic Animals in Brazil Provide New Insights into Parvovirus Distribution and Diversity: Viruses, v. 10.
Derakhshani, H., K. B. Fehr, S. Sepehri, D. Francoz, J. De Buck, H. W. Barkema, J. C. Plaizier, and E. Khafipour, 2018a, Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility: J Dairy Sci, v. 101, p. 10605-10625.
Derakhshani, H., J. C. Plaizier, J. De Buck, H. W. Barkema, and E. Khafipour, 2018b, Composition of the teat canal and intramammary microbiota of dairy cows subjected to antimicrobial dry cow therapy and internal teat sealant: J Dairy Sci, v. 101, p. 10191-10205.
Di Martino, B., F. Di Profio, E. Di Felice, C. Ceci, M. G. Pistilli, and F. Marsilio, 2012, Molecular detection of bovine kobuviruses in Italy: Arch Virol, v. 157, p. 2393-6.
Dowd, S. E., T. R. Callaway, R. D. Wolcott, Y. Sun, T. McKeehan, R. G. Hagevoort, and T. S. Edrington, 2008, Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP): BMC Microbiol, v. 8, p. 125.
Doyle, C. J., D. Gleeson, P. W. O'Toole, and P. D. Cotter, 2017, Impacts of Seasonal Housing and Teat Preparation on Raw Milk Microbiota: a High-Throughput Sequencing Study: Appl Environ Microbiol, v. 83.
Durso, L. M., G. P. Harhay, T. P. Smith, J. L. Bono, T. Z. Desantis, D. M. Harhay, G. L. Andersen, J. E. Keen, W. W. Laegreid, and M. L. Clawson, 2010, Animal-to-animal variation in fecal microbial diversity among beef cattle: Appl Environ Microbiol, v. 76, p. 4858-62.
Durso, L. M., J. E. Wells, G. P. Harhay, W. C. Rice, L. Kuehn, J. L. Bono, S. Shackelford, T. Wheeler, and T. P. Smith, 2012, Comparison of bacterial communities in faeces of beef cattle fed diets containing corn and wet distillers' grain with solubles: Lett Appl Microbiol, v. 55, p. 109-14.
Díez-Villaseñor, C., and F. Rodriguez-Valera, 2019, CRISPR analysis suggests that small circular single-stranded DNA smacoviruses infect Archaea instead of humans: Nat Commun, v. 10, p. 294.
Edgar, R. C., 2013, UPARSE: highly accurate OTU sequences from microbial amplicon reads: Nat Methods, v. 10, p. 996-8.
Ercolini, D., F. Russo, A. Nasi, P. Ferranti, and F. Villani, 2009, Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef: Appl Environ Microbiol, v. 75, p. 1990-2001.
Falentin, H., L. Rault, A. Nicolas, D. S. Bouchard, J. Lassalas, P. Lamberton, J. M. Aubry, P. G. Marnet, Y. Le Loir, and S. Even, 2016, Bovine Teat Microbiome Analysis Revealed Reduced Alpha Diversity and Significant Changes in Taxonomic Profiles in Quarters with a History of Mastitis: Front Microbiol, v. 7, p. 480.
Federal Food Safety and Veterinary Office, S., 2015, Tiergesundheitsbericht 2015.
Federal Food Safety and Veterinary Office, S., 2018, Bericht zur Überwachung von Tierseuchen.

Fulton, R. W., J. M. d'Offay, C. Landis, D. G. Miles, R. A. Smith, J. T. Saliki, J. F. Ridpath, A. W. Confer, J. D. Neill, R. Eberle, T. J. Clement, C. C. Chase, L. J. Burge, and M. E. Payton, 2016, Detection and characterization of viruses as field and vaccine strains in feedlot cattle with bovine respiratory disease: Vaccine, v. 34, p. 3478-92.

Gaeta, N. C., S. F. Lima, A. G. Teixeira, E. K. Ganda, G. Oikonomou, L. Gregory, and R. C. Bicalho, 2017, Deciphering upper respiratory tract microbiota complexity in healthy calves and calves that develop respiratory disease using shotgun metagenomics: J Dairy Sci, v. 100, p. 1445-1458.

Galvao, K. N., R. C. Bicalho, and S. J. Jeon, 2019, Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows: J Dairy Sci, v. 102, p. 11786-11797.

Galvão, K. N., R. C. Bicalho, and S. J. Jeon, 2019, Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows: J Dairy Sci, v. 102, p. 11786-11797.

Ganda, E. K., R. S. Bisinotto, S. F. Lima, K. Kronauer, D. H. Decter, G. Oikonomou, Y. H. Schukken, and R. C. Bicalho, 2016, Longitudinal metagenomic profiling of bovine milk to assess the impact of intramammary treatment using a third-generation cephalosporin: Sci Rep, v. 6, p. 37565.

Ganda, E. K., N. Gaeta, A. Sipka, B. Pomeroy, G. Oikonomou, Y. H. Schukken, and R. C. Bicalho, 2017, Normal milk microbiome is reestablished following experimental infection with Escherichia coli independent of intramammary antibiotic treatment with a third-generation cephalosporin in bovines: Microbiome, v. 5, p. 74.

Gates, M. C., M. E. Woolhouse, G. J. Gunn, and R. W. Humphry, 2013, Relative associations of cattle movements, local spread, and biosecurity with bovine viral diarrhoea virus (BVDV) seropositivity in beef and dairy herds: Prev Vet Med, v. 112, p. 285-95.

Gomez, D. E., L. G. Arroyo, M. C. Costa, L. Viel, and J. S. Weese, 2017, Characterization of the Fecal Bacterial Microbiota of Healthy and Diarrheic Dairy Calves: J Vet Intern Med, v. 31, p. 928-939.

Greenleaf, W. J., and S. M. Block, 2006, Single-molecule, motion-based DNA sequencing using RNA polymerase: Science, v. 313, p. 801.

Griffin, D., M. M. Chengappa, J. Kuszak, and D. S. McVey, 2010, Bacterial pathogens of the bovine respiratory disease complex: Vet Clin North Am Food Anim Pract, v. 26, p. 381-94.

Gruninger, R. J., G. O. Ribeiro, A. Cameron, and T. A. McAllister, 2019, Invited review: Application of meta-omics to understand the dynamic nature of the rumen microbiome and how it responds to diet in ruminants: Animal, v. 13, p. 1843-1854.

Guo, Z., Q. He, C. Tang, B. Zhang, and H. Yue, 2018, Identification and genomic characterization of a novel CRESS DNA virus from a calf with severe hemorrhagic enteritis in China: Virus Res, v. 255, p. 141-146.

Hagey, J. V., S. Bhatnagar, J. M. Heguy, B. M. Karle, P. L. Price, D. Meyer, and E. A. Maga, 2019, Fecal Microbial Communities in a Large Representative Cohort of California Dairy Cows: Front Microbiol, v. 10, p. 1093.

Hagi, T., K. Sasaki, H. Aso, and M. Nomura, 2013, Adhesive properties of predominant bacteria in raw cow's milk to bovine mammary gland epithelial cells: Folia Microbiol (Praha), v. 58, p. 515-22.

Hall, R. J., M. Leblanc-Maridor, J. Wang, X. Ren, N. E. Moore, C. R. Brooks, M. Peacey, J. Douwes, and D. J. McLean, 2013, Metagenomic detection of viruses in aerosol samples from workers in animal slaughterhouses: PLoS One, v. 8, p. e72226.

Harris, T. D., P. R. Buzby, H. Babcock, E. Beer, J. Bowers, I. Braslavsky, M. Causey, J. Colonell, J. Dimeo, J. W. Efcavitch, E. Giladi, J. Gill, J. Healy, M. Jarosz, D. Lapen, K. Moulton, S. R. Quake, K. Steinmann, E. Thayer, A. Tyurina, R. Ward, H. Weiss,

and Z. Xie, 2008, Single-molecule DNA sequencing of a viral genome: Science, v. 320, p. 106-9.
Hause, B. M., E. A. Collin, J. Anderson, R. A. Hesse, and G. Anderson, 2015, Bovine rhinitis viruses are common in U.S. cattle with bovine respiratory disease: PLoS One, v. 10, p. e0121998.
He, H., J. Dong, C. N. Lee, and Y. Li, 2009, Molecular analysis of spoilage-related bacteria in pasteurized milk during refrigeration by PCR and denaturing gradient gel electrophoresis: J Food Prot, v. 72, p. 572-7.
Heather, J. M., and B. Chain, 2016, The sequence of sequencers: The history of sequencing DNA: Genomics, v. 107, p. 1-8.
Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young, P. H. Janssen, and G. R. C. Collaborators, 2015, Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range: Sci Rep, v. 5, p. 14567.
Herlemann, D. P., M. Labrenz, K. Jürgens, S. Bertilsson, J. J. Waniek, and A. F. Andersson, 2011, Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea: ISME J, v. 5, p. 1571-9.
Hoffmann, B., M. Scheuch, D. Höper, R. Jungblut, M. Holsteg, H. Schirrmeier, M. Eschbaumer, K. V. Goller, K. Wernike, M. Fischer, A. Breithaupt, T. C. Mettenleiter, and M. Beer, 2012, Novel orthobunyavirus in Cattle, Europe, 2011: Emerg Infect Dis, v. 18, p. 469-72.
Holman, D. B., T. A. McAllister, E. Topp, A. D. Wright, and T. W. Alexander, 2015a, The nasopharyngeal microbiota of feedlot cattle that develop bovine respiratory disease: Vet Microbiol, v. 180, p. 90-5.
Holman, D. B., E. Timsit, and T. W. Alexander, 2015b, The nasopharyngeal microbiota of feedlot cattle: Sci Rep, v. 5, p. 15557.
Holman, D. B., E. Timsit, S. Amat, D. W. Abbott, A. G. Buret, and T. W. Alexander, 2017, The nasopharyngeal microbiota of beef cattle before and after transport to a feedlot: BMC Microbiol, v. 17, p. 70.
Hoque, M. N., A. Istiaq, R. A. Clement, M. Sultana, K. A. Crandall, A. Z. Siddiki, and M. A. Hossain, 2019, Metagenomic deep sequencing reveals association of microbiome signature with functional biases in bovine mastitis: Sci Rep, v. 9, p. 13536.
https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/686/genus-kobuvirus, December 16, 2019.
https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae/1041/genus-bocaparvovirus, July 26, 2020.
https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=20132565&src=NCBI&ictv_id=20132565, July 26, 2020.
https://www.agrarbericht.ch/de/betrieb/strukturen/soemmerungsbetriebe, April 29, 2020.
https://www.bfs.admin.ch/bfs/de/home/aktuell/neue-veroeffentlichungen.assetdetail.8467547.html, November 25, 2019.
https://www.bfs.admin.ch/bfs/de/home/aktuell/neue-veroeffentlichungen.assetdetail.9927607.html, November 25, 2019.
https://www.bfs.admin.ch/bfs/en/home/statistics/agriculture-forestry/farming.html, November 19, 2019.
https://www.census.gov/popclock/, March 30, 2020.
Hu, X., S. Li, Y. Fu, and N. Zhang, 2019, Targeting gut microbiota as a possible therapy for mastitis: Eur J Clin Microbiol Infect Dis, v. 38, p. 1409-1423.
Hunkapiller, T., R. J. Kaiser, B. F. Koop, and L. Hood, 1991, Large-scale and automated DNA sequence determination: Science, v. 254, p. 59-67.
Hyman, E. D., 1988, A new method of sequencing DNA: Anal Biochem, v. 174, p. 423-36.

Hässig, M., S. K. Spillmann, and P. Rüsch, 1988, [Serologic studies of the distribution of bovine parvovirus in Switzerland]: Schweiz Arch Tierheilkd, v. 130, p. 613-9.
Jackova, A., I. Sliz, R. Mandelik, S. Salamunova, J. Novotny, M. Kolesarova, M. Vlasakova, and S. Vilcek, 2017, Porcine kobuvirus 1 in healthy and diarrheic pigs: Genetic detection and characterization of virus and co-infection with rotavirus A: Infect Genet Evol, v. 49, p. 73-77.
Jeoung, H. Y., J. A. Lim, W. Jeong, J. K. Oem, and D. J. An, 2011, Three clusters of bovine kobuvirus isolated in Korea, 2008-2010: Virus Genes, v. 42, p. 402-6.
Jia, H., Y. Guo, W. Zhao, and K. Wang, 2014, Long-range PCR in next-generation sequencing: comparison of six enzymes and evaluation on the MiSeq sequencer: Sci Rep, v. 4, p. 5737.
Johnson, K. K., and D. L. Pendell, 2017, Market Impacts of Reducing the Prevalence of Bovine Respiratory Disease in United States Beef Cattle Feedlots: Front Vet Sci, v. 4, p. 189.
Johnston, D., B. Earley, P. Cormican, G. Murray, D. A. Kenny, S. M. Waters, M. McGee, A. K. Kelly, and M. S. McCabe, 2017, Illumina MiSeq 16S amplicon sequence analysis of bovine respiratory disease associated bacteria in lung and mediastinal lymph node tissue: BMC Vet Res, v. 13, p. 118.
Kaszab, E., A. Doszpoly, G. Lanave, A. Verma, K. Bányai, Y. S. Malik, and S. Marton, 2020, Chapter 2 - Metagenomics revealing new virus species in farm and pet animals and aquaculture, *in* Y. S. Malik, D. Barh, V. Azevedo, and S. M. P. Khurana, eds., Genomics and Biotechnological Advances in Veterinary, Poultry, and Fisheries, Academic Press, p. 29-73.
Khamrin, P., N. Maneekarn, S. Peerakome, S. Okitsu, M. Mizuguchi, and H. Ushijima, 2008, Bovine kobuviruses from cattle with diarrhea: Emerg Infect Dis, v. 14, p. 985-6.
Kim, H. K., S. J. Park, V. G. Nguyen, D. S. Song, H. J. Moon, B. K. Kang, and B. K. Park, 2012, Identification of a novel single-stranded, circular DNA virus from bovine stool: J Gen Virol, v. 93, p. 635-9.
Kim, M., J. Kim, L. A. Kuehn, J. L. Bono, E. D. Berry, N. Kalchayanand, H. C. Freetly, A. K. Benson, and J. E. Wells, 2014, Investigation of bacterial diversity in the feces of cattle fed different diets: J Anim Sci, v. 92, p. 683-94.
Kim, M., T. Park, and Z. Yu, 2017, Metagenomic investigation of gastrointestinal microbiome in cattle: Asian-Australas J Anim Sci, v. 30, p. 1515-1528.
Klima, C. L., D. B. Holman, B. J. Ralston, K. Stanford, R. Zaheer, T. W. Alexander, and T. A. McAllister, 2019, Lower Respiratory Tract Microbiome and Resistome of Bovine Respiratory Disease Mortalities: Microb Ecol, v. 78, p. 446-456.
Kraberger, S., K. Waits, J. Ivan, E. Newkirk, S. VandeWoude, and A. Varsani, 2018, Identification of circular single-stranded DNA viruses in faecal samples of Canada lynx (Lynx canadensis), moose (Alces alces) and snowshoe hare (Lepus americanus) inhabiting the Colorado San Juan Mountains: Infect Genet Evol, v. 64, p. 1-8.
Krupovic, M., A. Varsani, D. Kazlauskas, M. Breitbart, E. Delwart, K. Rosario, N. Yutin, Y. I. Wolf, B. Harrach, F. M. Zerbini, V. V. Dolja, J. H. Kuhn, and E. V. Koonin, 2020, : a Virus Phylum Unifying Seven Families of Rep-Encoding Viruses with Single-Stranded, Circular DNA Genomes: J Virol, v. 94.
Kuehn, J. S., P. J. Gorden, D. Munro, R. Rong, Q. Dong, P. J. Plummer, C. Wang, and G. J. Phillips, 2013, Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis: PLoS One, v. 8, p. e61959.
Kumar, K. R., M. J. Cowley, and R. L. Davis, 2019, Next-Generation Sequencing and Emerging Technologies: Semin Thromb Hemost, v. 45, p. 661-673.
Kursa, M. B., and W. R. Rudnicki, 2010, Feature Selection with the Boruta Package: 2010, v. 36, p. 13.

Kwok, K. T. T., D. F. Nieuwenhuijse, M. V. T. Phan, and M. P. G. Koopmans, 2020, Virus Metagenomics in Farm Animals: A Systematic Review: Viruses, v. 12.
Kälbergesundheitsdienst, S., 2018, Geschäftsbericht KGD.
Lauková, A., M. Pogány Simonová, I. Kubašová, R. Miltko, G. Bełżecki, and V. Strompfová, 2018, Sensitivity to antimicrobials of faecal Buttiauxella spp. from roe and red deer (Capreolus capreolus, Cervus elaphus)detected with MALDI-TOF mass spectrometry: Pol J Vet Sci, v. 21, p. 543-547.
Li, L., S. Diab, S. McGraw, B. Barr, R. Traslavina, R. Higgins, T. Talbot, P. Blanchard, G. Rimoldi, E. Fahsbender, B. Page, T. G. Phan, C. Wang, X. Deng, P. Pesavento, and E. Delwart, 2013, Divergent astrovirus associated with neurologic disease in cattle: Emerg Infect Dis, v. 19, p. 1385-92.
Lima, S. F., A. G. Teixeira, C. H. Higgins, F. S. Lima, and R. C. Bicalho, 2016, The upper respiratory tract microbiome and its potential role in bovine respiratory disease and otitis media: Sci Rep, v. 6, p. 29050.
Ling, Y., X. Zhang, G. Qi, S. Yang, L. Jingjiao, Q. Shen, X. Wang, L. Cui, X. Hua, X. Deng, E. Delwart, and W. Zhang, 2019, Viral metagenomics reveals significant viruses in the genital tract of apparently healthy dairy cows: Arch Virol, v. 164, p. 1059-1067.
Love, M. I., W. Huber, and S. Anders, 2014, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2: Genome Biol, v. 15, p. 550.
Machado, V. S., G. Oikonomou, M. L. Bicalho, W. A. Knauer, R. Gilbert, and R. C. Bicalho, 2012, Investigation of postpartum dairy cows' uterine microbial diversity using metagenomic pyrosequencing of the 16S rRNA gene: Vet Microbiol, v. 159, p. 460-9.
Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik, 2019, cluster: Cluster Analysis Basics and Extensions. R package version 2.0.9.
Magurran, A. E., 2004, Measuring biological diversity: Malden, Mass., Blackwell Science, 256 S. p.
Manteufel, J., and U. Truyen, 2008, Animal bocaviruses: a brief review: Intervirology, v. 51, p. 328-34.
Mao, S., M. Zhang, J. Liu, and W. Zhu, 2015, Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function: Sci Rep, v. 5, p. 16116.
Mao, S., R. Zhang, D. Wang, and W. Zhu, 2012, The diversity of the fecal bacterial community and its relationship with the concentration of volatile fatty acids in the feces during subacute rumen acidosis in dairy cows: BMC Vet Res, v. 8, p. 237.
Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg, 2005, Genome sequencing in microfabricated high-density picolitre reactors: Nature, v. 437, p. 376-80.
Martins, M. L., U. M. Pinto, K. Riedel, and M. C. D. Vanetti, 2018, Quorum Sensing and Spoilage Potential of Psychrotrophic Enterobacteriaceae Isolated from Milk: Biomed Res Int, v. 2018, p. 2723157.
Masuda, T., M. Nagai, H. Yamasato, S. Tsuchiaka, S. Okazaki, Y. Katayama, M. Oba, N. Nishiura, Y. Sassa, T. Omatsu, T. Furuya, S. Koyama, J. Shirai, K. Taniguchi, Y. Fujii, R. Todaka, K. Katayama, and T. Mizutani, 2014, Identification of novel bovine

group A rotavirus G15P[14] strain from epizootic diarrhea of adult cows by de novo sequencing using a next-generation sequencer: Vet Microbiol, v. 171, p. 66-73.
Matthews, C., F. Crispie, E. Lewis, M. Reid, P. W. O'Toole, and P. D. Cotter, 2019, The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency: Gut Microbes, v. 10, p. 115-132.
Mauroy, A., A. Scipioni, E. Mathijs, C. Thys, and E. Thiry, 2009, Molecular detection of kobuviruses and recombinant noroviruses in cattle in continental Europe: Arch Virol, v. 154, p. 1841-5.
McDaneld, T. G., L. A. Kuehn, and J. W. Keele, 2018, Evaluating the microbiome of two sampling locations in the nasal cavity of cattle with bovine respiratory disease complex (BRDC): J Anim Sci.
McGarvey, J. A., S. W. Hamilton, E. J. DePeters, and F. M. Mitloehner, 2010, Effect of dietary monensin on the bacterial population structure of dairy cattle colonic contents: Appl Microbiol Biotechnol, v. 85, p. 1947-52.
McMullen, C., K. Orsel, T. W. Alexander, F. van der Meer, G. Plastow, and E. Timsit, 2018, Evolution of the nasopharyngeal bacterial microbiota of beef calves from spring processing to 40 days after feedlot arrival: Vet Microbiol, v. 225, p. 139-148.
McMullen, C., K. Orsel, T. W. Alexander, F. van der Meer, G. Plastow, and E. Timsit, 2019, Comparison of the nasopharyngeal bacterial microbiota of beef calves raised without the use of antimicrobials between healthy calves and those diagnosed with bovine respiratory disease: Vet Microbiol, v. 231, p. 56-62.
Metzger, S. A., L. L. Hernandez, G. Suen, and P. L. Ruegg, 2018, Understanding the Milk Microbiota: Vet Clin North Am Food Anim Pract, v. 34, p. 427-438.
Miranda-CasoLuengo, R., J. Lu, E. J. Williams, A. A. Miranda-CasoLuengo, S. D. Carrington, A. C. O. Evans, and W. G. Meijer, 2019, Delayed differentiation of vaginal and uterine microbiomes in dairy cows developing postpartum endometritis: PLoS One, v. 14, p. e0200974.
Mitra, N., N. Cernicchiaro, S. Torres, F. Li, and B. M. Hause, 2016, Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus: J Gen Virol, v. 97, p. 1771-84.
Mohamed, F. F., S. M. G. Mansour, A. Orabi, I. E. El-Araby, T. F. F. Ng, S. K. Mor, and S. M. Goyal, 2018, Detection and genetic characterization of bovine kobuvirus from calves in Egypt: Arch Virol, v. 163, p. 1439-1447.
Moore, S. G., A. C. Ericsson, S. E. Poock, P. Melendez, and M. C. Lucy, 2017, Hot topic: 16S rRNA gene sequencing reveals the microbiome of the virgin and pregnant bovine uterus: J Dairy Sci, v. 100, p. 4953-4960.
Moore, S. J., M. A. O'Dea, N. Perkins, and A. J. O'Hara, 2015, Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle: J Vet Diagn Invest, v. 27, p. 6-17.
Moreira, A. S., S. M. Raabis, M. E. Graham, J. M. Dreyfus, S. D. Sibley, J. A. Godhardt-Cooper, K. L. Toohey-Kurth, T. L. Goldberg, and S. F. Peek, 2017, Identification by next-generation sequencing of Aichivirus B in a calf with enterocolitis and neurologic signs: J Vet Diagn Invest, v. 29, p. 208-211.
Myer, P. R., J. E. Wells, T. P. Smith, L. A. Kuehn, and H. C. Freetly, 2015, Microbial community profiles of the colon from steers differing in feed efficiency: Springerplus, v. 4, p. 454.
Nagai, M., T. Omatsu, H. Aoki, Y. Kaku, G. J. Belsham, K. Haga, Y. Naoi, K. Sano, M. Umetsu, M. Shiokawa, S. Tsuchiaka, T. Furuya, S. Okazaki, Y. Katayama, M. Oba, J. Shirai, K. Katayama, and T. Mizutani, 2015a, Identification and complete genome analysis of a novel bovine picornavirus in Japan: Virus Res, v. 210, p. 205-12.

Nagai, M., T. Omatsu, H. Aoki, K. Otomaru, T. Uto, M. Koizumi, F. Minami-Fukuda, H. Takai, T. Murakami, T. Masuda, H. Yamasato, M. Shiokawa, S. Tsuchiaka, Y. Naoi, K. Sano, S. Okazaki, Y. Katayama, M. Oba, T. Furuya, J. Shirai, and T. Mizutani, 2015b, Full genome analysis of bovine astrovirus from fecal samples of cattle in Japan: identification of possible interspecies transmission of bovine astrovirus: Arch Virol, v. 160, p. 2491-501.

Nantel-Fortier, N., V. Lachapelle, A. Letellier, Y. L'Homme, and J. Brassard, 2019, Kobuvirus shedding dynamics in a swine production system and their association with diarrhea: Vet Microbiol, v. 235, p. 319-326.

Ng, T. F., N. O. Kondov, X. Deng, A. Van Eenennaam, H. L. Neibergs, and E. Delwart, 2015a, A metagenomics and case-control study to identify viruses associated with bovine respiratory disease: J Virol, v. 89, p. 5340-9.

Ng, T. F., W. Zhang, J. Sachsenröder, N. O. Kondov, A. C. da Costa, E. Vega, L. R. Holtz, G. Wu, D. Wang, C. O. Stine, M. Antonio, U. S. Mulvaney, M. O. Muench, X. Deng, K. Ambert-Balay, P. Pothier, J. Vinjé, and E. Delwart, 2015b, A diverse group of small circular ssDNA viral genomes in human and non-human primate stools: Virus Evol, v. 1, p. vev017.

Nicola, I., F. Cerutti, E. Grego, I. Bertone, P. Gianella, A. D'Angelo, S. Peletto, and C. Bellino, 2017, Characterization of the upper and lower respiratory tract microbiota in Piedmontese calves: Microbiome, v. 5, p. 152.

Nyrén, P., 1987, Enzymatic method for continuous monitoring of DNA polymerase activity: Anal Biochem, v. 167, p. 235-8.

Nyrén, P., and A. Lundin, 1985, Enzymatic method for continuous monitoring of inorganic pyrophosphate synthesis: Anal Biochem, v. 151, p. 504-9.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, and et.al, 2017, V*egan: Community Ecology Package*.

Otomaru, K., Y. Naoi, K. Haga, T. Omatsu, T. Uto, M. Koizumi, T. Masuda, H. Yamasato, H. Takai, H. Aoki, S. Tsuchiaka, K. Sano, S. Okazaki, Y. Katayama, M. Oba, T. Furuya, J. Shirai, K. Katayama, T. Mizutani, and M. Nagai, 2016, Detection of novel kobu-like viruses in Japanese black cattle in Japan: J Vet Med Sci, v. 78, p. 321-4.

Ozutsumi, Y., H. Hayashi, M. Sakamoto, H. Itabashi, and Y. Benno, 2005, Culture-independent analysis of fecal microbiota in cattle: Biosci Biotechnol Biochem, v. 69, p. 1793-7.

Park, S. J., H. K. Kim, D. S. Song, H. J. Moon, and B. K. Park, 2011, Molecular detection and genetic characterization of kobuviruses in fecal samples collected from diarrheic cattle in Korea: Infect Genet Evol, v. 11, p. 1178-82.

Pearson, V. M., S. B. Caudle, and D. R. Rokyta, 2016, Viral recombination blurs taxonomic lines: examination of single-stranded DNA viruses in a wastewater treatment plant: PeerJ, v. 4, p. e2585.

Pielou, E. C., 1975, Ecological diversity: New York a.o., Wiley, VIII, 165 p. p.

Reuter, G., A. Boros, and P. Pankovics, 2011, Kobuviruses - a comprehensive review: Rev Med Virol, v. 21, p. 32-41.

Reuter, G., and L. Egyed, 2009, Bovine kobuvirus in europe: Emerg Infect Dis, v. 15, p. 822-3.

Reyes, G. R., and J. P. Kim, 1991, Sequence-independent, single-primer amplification (SISPA) of complex DNA populations: Mol Cell Probes, v. 5, p. 473-81.

Reynolds, A. P., G. Richards, B. de la Iglesia, and V. J. Rayward-Smith, 2006, Clustering Rules: A Comparison of Partitioning and Hierarchical Clustering Algorithms: Journal of Mathematical Modelling and Algorithms, v. 5, p. 475-504.

Ribeiro, J., E. Lorenzetti, A. F. Alfieri, and A. A. Alfieri, 2014, Kobuvirus (Aichivirus B) infection in Brazilian cattle herds: Vet Res Commun, v. 38, p. 177-82.

Ribeiro, J., E. Lorenzetti, J. C. R. Júnior, T. N. da Silva Medeiros, A. F. Alfieri, and A. A. Alfieri, 2017, Phylogenetic analysis of VP1 and RdRP genes of Brazilian aichivirus B strains involved in a diarrhea outbreak in dairy calves: Arch Virol, v. 162, p. 3691-3696.
Rice, W. C., M. L. Galyean, S. B. Cox, S. E. Dowd, and N. A. Cole, 2012, Influence of wet distillers grains diets on beef cattle fecal bacterial community structure: BMC Microbiol, v. 12, p. 25.
Ronaghi, M., S. Karamohamed, B. Pettersson, M. Uhlén, and P. Nyrén, 1996, Real-time DNA sequencing using detection of pyrophosphate release: Anal Biochem, v. 242, p. 84-9.
Ronaghi, M., M. Uhlén, and P. Nyrén, 1998, A sequencing method based on real-time pyrophosphate: Science, v. 281, p. 363, 365.
Rosario, K., S. Duffy, and M. Breitbart, 2012, A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics: Arch Virol, v. 157, p. 1851-71.
Ross, E. M., S. Petrovski, P. J. Moate, and B. J. Hayes, 2013, Metagenomics of rumen bacteriophage from thirteen lactating dairy cattle: BMC Microbiol, v. 13, p. 242.
Roux, S., M. Krupovic, A. Poulet, D. Debroas, and F. Enault, 2012, Evolution and diversity of the Microviridae viral family through a collection of 81 new complete genomes assembled from virome reads: PLoS One, v. 7, p. e40418.
Rudi, K., B. Moen, M. Sekelja, T. Frisli, and M. R. Lee, 2012, An eight-year investigation of bovine livestock fecal microbiota: Vet Microbiol, v. 160, p. 369-77.
Sadeghi, M., B. Kapusinszky, D. M. Yugo, T. G. Phan, X. Deng, I. Kanevsky, T. Opriessnig, A. R. Woolums, D. J. Hurley, X. J. Meng, and E. Delwart, 2017, Virome of US bovine calf serum: Biologicals, v. 46, p. 64-67.
Saikumar, G., and T. Das, 2019, Porcine Circovirus: Recent Advances in Animal Virology, p. 171-195.
Salter, S. J., M. J. Cox, E. M. Turek, S. T. Calus, W. O. Cookson, M. F. Moffatt, P. Turner, J. Parkhill, N. J. Loman, and A. W. Walker, 2014, Reagent and laboratory contamination can critically impact sequence-based microbiome analyses: BMC Biol, v. 12, p. 87.
Sandals, W. C., R. C. Povey, and A. H. Meek, 1995, Prevalence of bovine parvovirus infection in Ontario dairy cattle: Can J Vet Res, v. 59, p. 81-6.
Sanger, F., G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, C. A. Fiddes, C. A. Hutchison, P. M. Slocombe, and M. Smith, 1977, Nucleotide sequence of bacteriophage phi X174 DNA: Nature, v. 265, p. 687-95.
Savage, E., S. Chothe, V. Lintner, T. Pierre, T. Matthews, S. Kariyawasam, D. Miller, D. Tewari, and B. Jayarao, 2017, Evaluation of Three Bacterial Identification Systems for Species Identification of Bacteria Isolated from Bovine Mastitis and Bulk Tank Milk Samples: Foodborne Pathog Dis, v. 14, p. 177-187.
Schlottau, K., C. Schulze, S. Bilk, D. Hanke, D. Höper, M. Beer, and B. Hoffmann, 2016, Detection of a Novel Bovine Astrovirus in a Cow with Encephalitis: Transbound Emerg Dis, v. 63, p. 253-9.
Selbitz, H.-J., and G. Alber, 2011, Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre: Stuttgart, Enke, 710 S. p.
Shanks, O. C., C. A. Kelty, S. Archibeque, M. Jenkins, R. J. Newton, S. L. McLellan, S. M. Huse, and M. L. Sogin, 2011, Community structures of fecal bacteria in cattle from different animal feeding operations: Appl Environ Microbiol, v. 77, p. 2992-3001.
Sikorski, A., M. Massaro, S. Kraberger, L. M. Young, D. Smalley, D. P. Martin, and A. Varsani, 2013, Novel myco-like DNA viruses discovered in the faecal matter of various animals: Virus Res, v. 177, p. 209-16.
Steel, O., S. Kraberger, A. Sikorski, L. M. Young, R. J. Catchpole, A. J. Stevens, J. J. Ladley, D. S. Coray, D. Stainton, A. Dayaram, L. Julian, K. van Bysterveldt, and A. Varsani,

2016, Circular replication-associated protein encoding DNA viruses identified in the faecal matter of various animals in New Zealand: Infect Genet Evol, v. 43, p. 151-64.
Stein, L. D., 2010, The case for cloud computing in genome informatics: Genome Biol, v. 11, p. 207.
Stewart, R. D., M. D. Auffret, A. Warr, A. H. Wiser, M. O. Press, K. W. Langford, I. Liachko, T. J. Snelling, R. J. Dewhurst, A. W. Walker, R. Roehe, and M. Watson, 2018, Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen: Nat Commun, v. 9, p. 870.
Stroebel, C., T. Alexander, M. L. Workentine, and E. Timsit, 2018, Effects of transportation to and co-mingling at an auction market on nasopharyngeal and tracheal bacterial communities of recently weaned beef cattle: Vet Microbiol, v. 223, p. 126-133.
Sun, H.-Z., M. Xue, L. L. Guan, and J. Liu, 2019, A collection of rumen bacteriome data from 334 mid-lactation dairy cows: Scientific Data, v. 6, p. 180301.
Säde, E., A. Murros, and J. Björkroth, 2013, Predominant enterobacteria on modified-atmosphere packaged meat and poultry: Food Microbiol, v. 34, p. 252-8.
Tang, M. T., H. Han, Z. Yu, T. Tsuruta, and N. Nishino, 2017, Variability, stability, and resilience of fecal microbiota in dairy cows fed whole crop corn silage: Appl Microbiol Biotechnol, v. 101, p. 6355-6364.
Taponen, S., D. McGuinness, H. Hiitiö, H. Simojoki, R. Zadoks, and S. Pyörälä, 2019, Bovine milk microbiome: a more complex issue than expected: Vet Res, v. 50, p. 44.
Thomas, M., M. Webb, S. Ghimire, A. Blair, K. Olson, G. J. Fenske, A. T. Fonder, J. Christopher-Hennings, D. Brake, and J. Scaria, 2017, Metagenomic characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle: Sci Rep, v. 7, p. 12257.
Timsit, E., M. Workentine, A. B. Schryvers, D. B. Holman, F. van der Meer, and T. W. Alexander, 2016, Evolution of the nasopharyngeal microbiota of beef cattle from weaning to 40 days after arrival at a feedlot: Vet Microbiol, v. 187, p. 75-81.
Timsit, E., M. Workentine, F. van der Meer, and T. Alexander, 2018, Distinct bacterial metacommunities inhabit the upper and lower respiratory tracts of healthy feedlot cattle and those diagnosed with bronchopneumonia: Vet Microbiol, v. 221, p. 105-113.
Tisza, M. J., D. V. Pastrana, N. L. Welch, B. Stewart, A. Peretti, G. J. Starrett, Y. S. Pang, S. R. Krishnamurthy, P. A. Pesavento, D. H. McDermott, P. M. Murphy, J. L. Whited, B. Miller, J. Brenchley, S. P. Rosshart, B. Rehermann, J. Doorbar, B. A. Ta'ala, O. Pletnikova, J. C. Troncoso, S. M. Resnick, B. Bolduc, M. B. Sullivan, A. Varsani, A. M. Segall, and C. B. Buck, 2020, Discovery of several thousand highly diverse circular DNA viruses: Elife, v. 9.
Tokarz, R., S. Sameroff, R. A. Hesse, B. M. Hause, A. Desai, K. Jain, and W. Ian Lipkin, 2015, Discovery of a novel nidovirus in cattle with respiratory disease: J Gen Virol, v. 96, p. 2188-2193.
Tong, J., H. Zhang, Y. Zhang, B. Xiong, and L. Jiang, 2019, Microbiome and Metabolome Analyses of Milk From Dairy Cows With Subclinical: Front Microbiol, v. 10, p. 2547.
USDA, N. A. S. S., 2018, Cattle (January 2018).
USDA–APHIS–VS, 2013, National Animal Health Monitoring System Beef Feedlot Study 2011. Part IV: Health and Health Management on U.S. Feedlots with a Capacity of 1,000 or More Head.
van Dijk, E. L., H. Auger, Y. Jaszczyszyn, and C. Thermes, 2014, Ten years of next-generation sequencing technology: Trends Genet, v. 30, p. 418-26.
Varsani, A., and M. Krupovic, 2018, Smacoviridae: a new family of animal-associated single-stranded DNA viruses: Arch Virol, v. 163, p. 2005-2015.

Wang, H., S. Li, A. Mahmood, S. Yang, X. Wang, Q. Shen, T. Shan, X. Deng, J. Li, X. Hua, L. Cui, E. Delwart, and W. Zhang, 2018a, Plasma virome of cattle from forest region revealed diverse small circular ssDNA viral genomes: Virol J, v. 15, p. 11.
Wang, Y., J. Wang, H. Li, K. Fu, B. Pang, Y. Yang, Y. Liu, W. Tian, and R. Cao, 2018b, Characterization of the cervical bacterial community in dairy cows with metritis and during different physiological phases: Theriogenology, v. 108, p. 306-313.
Watson, J. D., and F. H. Crick, 1953, Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid: Nature, v. 171, p. 737-8.
Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue, 2002, Phylogenies and community ecology: Annual review of ecology and systematics, v. 33, p. 475-505.
Wong, K., T. I. Shaw, A. Oladeinde, T. C. Glenn, B. Oakley, and M. Molina, 2016, Rapid Microbiome Changes in Freshly Deposited Cow Feces under Field Conditions: Front Microbiol, v. 7, p. 500.
Woo, P. C., S. K. Lau, J. L. Teng, A. K. Tsang, M. Joseph, E. Y. Wong, Y. Tang, S. Sivakumar, R. Bai, R. Wernery, U. Wernery, and K. Y. Yuen, 2014, Metagenomic analysis of viromes of dromedary camel fecal samples reveals large number and high diversity of circoviruses and picobirnaviruses: Virology, v. 471-473, p. 117-25.
Wüthrich, D., C. L. Boujon, L. Truchet, S. Selimovic-Hamza, A. Oevermann, I. G. Bouzalas, R. Bruggmann, and T. Seuberlich, 2016, Exploring the virome of cattle with non-suppurative encephalitis of unknown etiology by metagenomics: Virology, v. 493, p. 22-30.
Yamashita, T., M. Ito, Y. Kabashima, H. Tsuzuki, A. Fujiura, and K. Sakae, 2003, Isolation and characterization of a new species of kobuvirus associated with cattle: J Gen Virol, v. 84, p. 3069-77.
Yanai, I., H. Benjamin, M. Shmoish, V. Chalifa-Caspi, M. Shklar, R. Ophir, A. Bar-Even, S. Horn-Saban, M. Safran, E. Domany, D. Lancet, and O. Shmueli, 2005, Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification: Bioinformatics, v. 21, p. 650-9.
Zaheer, R., S. M. Lakin, R. O. Polo, S. R. Cook, F. J. Larney, P. S. Morley, C. W. Booker, S. J. Hannon, G. Van Domselaar, R. R. Read, and T. A. McAllister, 2019, Comparative diversity of microbiomes and Resistomes in beef feedlots, downstream environments and urban sewage influent: BMC Microbiol, v. 19, p. 197.
Zeineldin, M., J. Lowe, and B. Aldridge, 2019, Contribution of the Mucosal Microbiota to Bovine Respiratory Health: Trends Microbiol, v. 27, p. 753-770.
Zeineldin, M., J. Lowe, M. de Godoy, N. Maradiaga, C. Ramirez, M. Ghanem, Y. Abd El-Raof, and B. Aldridge, 2017a, Disparity in the nasopharyngeal microbiota between healthy cattle on feed, at entry processing and with respiratory disease: Vet Microbiol, v. 208, p. 30-37.
Zeineldin, M. M., J. F. Lowe, E. D. Grimmer, M. R. C. de Godoy, M. M. Ghanem, Y. M. Abd El-Raof, and B. M. Aldridge, 2017b, Relationship between nasopharyngeal and bronchoalveolar microbial communities in clinically healthy feedlot cattle: BMC Microbiol, v. 17, p. 138.
Zhan, J., M. Liu, C. Wu, X. Su, K. Zhan, and G. Q. Zhao, 2017, Effects of alfalfa flavonoids extract on the microbial flora of dairy cow rumen: Asian-Australas J Anim Sci, v. 30, p. 1261-1269.
Zhang, H., E. Porter, M. Lohman, N. Lu, L. Peddireddi, G. Hanzlicek, D. Marthaler, X. Liu, and J. Bai, 2018, Influenza C Virus in Cattle with Respiratory Disease, United States, 2016-2018: Emerg Infect Dis, v. 24, p. 1926-1929.
Zhang, M., J. E. Hill, C. Fernando, T. W. Alexander, E. Timsit, F. van der Meer, and Y. Huang, 2019a, Respiratory viruses identified in western Canadian beef cattle by

metagenomic sequencing and their association with bovine respiratory disease: Transbound Emerg Dis, v. 66, p. 1379-1386.
Zhang, M., J. E. Hill, D. L. Godson, M. Ngeleka, C. Fernando, and Y. Huang, 2019b, The pulmonary virome, bacteriological and histopathological findings in bovine respiratory disease from western Canada: Transbound Emerg Dis.
Zhao, L., K. Rosario, M. Breitbart, and S. Duffy, 2019, Chapter Three - Eukaryotic Circular Rep-Encoding Single-Stranded DNA (CRESS DNA) Viruses: Ubiquitous Viruses With Small Genomes and a Diverse Host Range, *in* M. Kielian, T. C. Mettenleiter, and M. J. Roossinck, eds., Advances in Virus Research, v. 103, Academic Press, p. 71-133.

Acknowledgements

First, I would like to thank PD Dr. Claudia Bachofen for giving me the opportunity to conduct my dissertation under her supervision and in her research group. I specially would like to thank her for the good advice and great support.

Further I like to thank Prof. Dr. Cornel Fraefel for the possibility to work in his institute and his scientific advice.

I thank Prof. Dr. Michael Hässig for co-examining my dissertation and his support in organising the sample collection.
I would like to thank the team of the AgroVet-Strickhof facility for helping me collect the samples.

I further thank Dr. Marc Schmid for analysing the bacteriome results and his scientific advice.

I thank the team of the Functional Genomics Center Zurich for their technical and scientific support.

I thank all members of the institute for their support and advice.

A very special thanks goes to Charlotte, Isabelle, Jakub, Deborah, Isabelle, Tamara, Julia, Marco and Martina for the amazing atmosphere in the lab, the teamwork, the good discussions, the moral support and last but not least the many laughs.

At last, a big thanks to all my family and friends, especially Toby, for always supporting me, for their good advice, their encouragement and patience.

This work was funded by the Institute of Virology, Vetsuisse Faculty, University of Zurich.

Curriculum Vitae

Vorname Name	Karin Meier
Geburtsdatum	10.06.1990
Geburtsort	Uster, Schweiz
Nationalität	Schweiz
Heimatort bei Schweizer/-in	Uster (ZH)
August 1997 – Juli 2003	Primarschule, Uster, Schweiz
August 2003 – September 2009	Gymnasium, Dübendorf, Schweiz
4. September 2009	Matura, altsprachliches Profil, Kantonsschule Glattal, Dübendorf, Schweiz
September 2011 – Dezember 2016	Studium der Veterinärmedizin, Universität Zürich, Schweiz
30. Dezember 2016	Staatsexamen med. vet., Universität Zürich, Schweiz
September 2017 – Dezember 2019	Anfertigung der Dissertation unter Leitung von PD Dr. med. vet. Claudia Bachofen am Virologischen Institut der Vetsuisse-Fakultät Universität Zürich Direktor Prof. Dr. sc. nat. ETH Cornel Fraefel